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Local Run Manager TruSight Oncology Comprehensive (EU) Analysis Module

Workflow Guide

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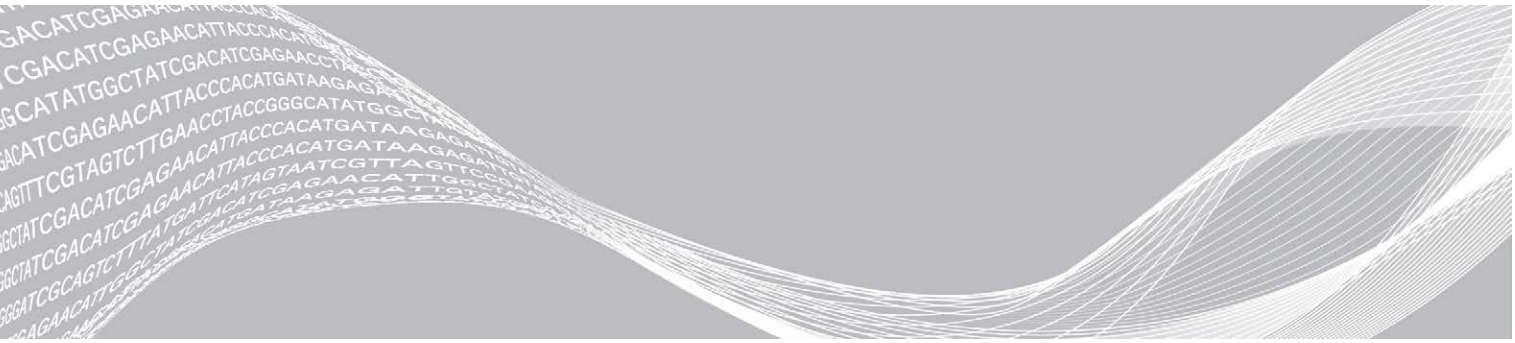

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Overview

The Illumina® Local Run Manager TruSight™ Oncology Comprehensive (EU) Analysis Module (TSO Comprehensive analysis module) analyzes sequencing reads of DNA and RNA libraries prepared using the TruSight Oncology Comprehensive (TSO Comprehensive) assay. The intended use for the TSO Comprehensive assay can be found in *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)*.

The TSO Comprehensive analysis module supports run setup, sequencing, analysis, and reporting for the prepared DNA and RNA libraries. For patient samples, the TSO Comprehensive analysis module generates:

- A TSO Comprehensive report for each patient sample, which includes companion diagnostic, tumor profiling, and quality control results (available in both PDF and JSON formats).
- A low depth report (*.tsv) for each patient sample, which 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), which includes analysis status and quality control metrics for all patient samples in a sequencing run.

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

The TSO Comprehensive (EU) Software Suite is used to install the TSO Comprehensive analysis module and supporting software components. The TSO Comprehensive (EU) Claims Package is installed into the TSO Comprehensive analysis module. For part numbers and version numbers, refer to *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)*.

About This Guide

This guide provides instructions for setting up run parameters for sequencing and analysis parameters for the TSO Comprehensive analysis module. Use of the software requires basic knowledge of the current Windows operating system and web browser-based user interface. For information about the Local Run Manager dashboard and system settings, refer to the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Enter Run Information

NextSeq 550Dx instrument Local Run Manager is the software used to set up a TSO Comprehensive assay run. For more information, refer to the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Enter run and sample setup information directly into the TSO Comprehensive analysis module.

Install a Knowledge Base

The TSO Comprehensive analysis module requires an installed Knowledge Base (KB) to perform analysis. KBs are available for download on the Illumina Lighthouse portal. Illumina periodically releases new KBs. To update the KB installed on the instrument, download the most recent KB that is compatible with your TSO Comprehensive analysis module. When updating a KB, the previously installed KB is removed during the installation process. A KB should not be installed while a sequencing run, analysis, or other installation process is in progress.

CAUTION

To avoid data loss, make sure that no other processes are in progress before following the installation instructions.

1 Download the desired KB (zip format) to a local directory on your instrument or a networked computer. Drive D is the preferred location.

2 Perform KB checksum verification as follows.

   a Perform a Windows search for PowerShell. Right-click on the program and select **Run as Administrator**.
   b Enter `Get-FileHash <KB file path>\<kbfilename.zip> -Algorithm MD5` in a PowerShell window to generate the MD5 checksum for the KB.
   c Compare the output MD5 checksum against the KB checksum from Illumina Lighthouse portal. If the checksums do not match, delete this KB file and redownload it from the portal.

3 Open Local Run Manager on your instrument or the networked computer (local area network). For more information on LRM user management, refer to *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

4 Sign in as an LRM admin or a non-admin user with permission to edit module settings.

5 Use the Tools menu to navigate to the Modules & Manifests screen.
The Modules & Manifests screen is referred to as Module Settings in TSO Comprehensive analysis module v2.3.3 and v2.3.6.

6 Select **TSO Comp (EU)**.

7 Select **Install New** under the Knowledge Base Version section of the screen.

8 An installation wizard prompts you to browse to the location of the KB zip file. Make sure that you are installing the KB downloaded in step 1.
The wizard also displays information about the KB including the name, version, RefSeq database version, and published date.

9 Select **Continue** in the installation wizard.
The installer verifies that the KB is compatible with the TSO Comprehensive analysis module and that the KB is not corrupt. It is not possible to launch a new TSO Comprehensive analysis while the KB is being installed.

CAUTION

Navigating away from the Modules & Manifests page or closing the browser while the KB is installing cancels the installation process.

10 After installation is complete, the new KB is displayed on the Modules & Manifests screen. The KB name and version are also displayed on the Create Run, Requeue Analysis, and Edit Run screens.

TSO Comprehensive Analysis Module Information

The TSO Comprehensive analysis module includes analysis module, KB, and claims package version information on the Modules & Manifests screen.

1 Open Local Run Manager on your instrument.

2 Use the Tools menu to navigate to the Modules & Manifests screen.

3 Select **TSO Comp (EU)**.

The Modules & Manifests screen displays the following installation information:

- **Device Identifier**—A unique device identifier for the installed TSO Comprehensive analysis module and associated Claims Package. This identifier is not impacted by the KB version installed.
- **Product Identifier**—The version of the installed TSO Comprehensive analysis module.
- **Modified On**—The date and time that the TSO Comprehensive analysis module itself was last installed or updated.
- **Sequencing Run Settings**—Displays the read type (paired-end) and read length settings associated with the TSO Comprehensive analysis module.
- **Claims Installed**—Displays the version of the installed Claims Package and associated Companion Diagnostic claims. The Claims Package includes the companion diagnostic intended use claims that will be evaluated by the TSO Comprehensive analysis module.
- **TSO Comprehensive Security Certificate**—For v2.3.5 and later (excluding v2.3.6), HTTPS certificate specific to this instrument that is required for remote access using a web browser of this instrument from another machine in the same network.
- **Knowledge Base Version**—Refer to *Install a Knowledge Base* on page 1 for instructions on installing or updating the KB. This section includes Knowledge Base installation information for the following fields:

Field	Description
Name	The KB name.
Version	The KB version.
RefSeq Version	The version of RefSeq included in the KB. When the RefSeq information originates from the Ensembl Variant Effect Predictor (VEP)[1] cache files, the VEP version is displayed.
Published	The date the KB was published.
Installed	The date the KB was installed.
State	The state of the KB installation. Will display as Ready when installation is completed.

[1] McLaren W, Gil L, Hunt SE, et al. The ensembl variant effect predictor. Genome Biol. 2016 Jun 6;17(1): 122.g

TSO Comprehensive assay v2.3.5 Security Certificate

The TSO Comprehensive analysis module uses HTTPS to encrypt data connections to make sure that run data is private and secure and is required for remote access of the instrument using a web browser from another machine in the same network. For version 2.3.5 and later (excluding v2.3.6), the TSO Comprehensive analysis module requires the installation of a TSO Comprehensive security certificate in addition to the NextSeq 550Dx instrument Local Run Manager security certificate.

For instructions on how to install the NextSeq 550Dx instrument Local Run Manager security certificate, refer to *Local Run Manager v2 Software Guide (document # 1000000002702)*.

To install the TSO Comprehensive security certificate, do as follows.

1 Open Local Run Manager on your instrument.

2 Use the Tools menu to navigate to the Modules & Manifests screen.

3 Select **TSO Comp (EU) module**.

4 Download the TSO Comp (EU) HTTPS Certificate.

5 Extract the contents of the zip file.

6 Right-click the BAT file and select **Run as administrator**.

7 Follow the prompts to finish the installation, and then restart your browser.

Regenerate Security Certificate

For version 2.3.5 and later (excluding v2.3.6), if there was recent change to the instrument name or the instrument was moved to a new domain, you must regenerate the security certificate to regain access to the NextSeq 550Dx instrument Local Run Manager and the TSO Comprehensive analysis module. For instructions on how to regenerate the NextSeq 550Dx instrumentLocal Run Manager security certificate, refer to *Local Run Manager v2 Software Guide (document # 1000000002702)*.

To regenerate the TSO Comprehensive security certificate, do as follows.

1 On the instrument, log in to the Windows operating system.

2 Using Windows File Explorer, navigate to the directory where the KB service is installed (eg, `C:\Illumina\Local Run Manager\Modules\TSOCompEU\[VersionNumber]\KBApiService\bin\Scripts`).

3 Right-click the BAT file and select **Run as administrator**.

4 Follow the prompts to finish the installation.

5 To connect to TSO Comprehensive analysis module from another device, download and install the regenerated certificate on the remote device.

Set Run Parameters

1 Log in to Local Run Manager on the instrument or from a networked computer.

2 Select **Create Run**, and then select **TSO Comp (EU)**.

3 Enter a run name that identifies the run from sequencing through analysis with the following criteria.
- 1–40 characters.
- Only alphanumeric characters, underscores, or dashes.
- Underscores and dashes must be preceded and followed by an alphanumeric character.
- Unique across all runs on the instrument.

4 **[Optional]** Enter a run description to help identify the run with the following criteria.
- 1–150 characters.
- Only alphanumeric characters or spaces.
- Spaces must be preceded and followed by an alphanumeric character.

Specify Samples for the Run

Specify samples for the run using one of the following options.

- **Enter samples manually**—Use the blank table on the Create Run screen. Refer to *the Number of Libraries and Selecting Indexes section in the TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)* for all the supported sample configurations.
- **Import samples**—Navigate to an external file in a comma-separated values (*.csv) format. A template is available for download on the Create Run screen.

CAUTION

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

CAUTION

To avoid data loss, make sure KB installation is not in progress before saving a run.

Enter Samples Manually

1 Enter a unique sample ID in the Sample ID field with the following criteria. **All control samples should be added first.** Refer to *Control Samples* on page 6 for more information.
- 1–25 characters.
- Only alphanumeric characters, underscores, or dashes.
- Underscores and dashes must be preceded and followed by an alphanumeric character.

2 **[Optional]** Enter a sample description in the Sample Description field with the following criteria.
- 1–50 characters.
- Only alphanumeric characters, dashes, underscores, or spaces.
- Spaces, underscores, and dashes must be preceded and followed by an alphanumeric character.

3 Select an index for the DNA library and/or RNA library prepared from the sample.
Make sure that RNA and DNA samples are in separate columns.
The DNA i7+i5 Sequence field auto-populates after selecting a DNA Index ID. The RNA i7+i5 Sequence field auto-populates after selecting an RNA Index ID.
In addition to the summary here, refer to the Number of Libraries and Selecting Indexes section in the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)* for index ID selection.
- For a DNA sample library, select a unique index ID (UPxx or CPxx indexes) from the DNA Index ID drop-down list.
- For an RNA sample library, select a unique index ID (UPxx only) from the RNA 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 (EU) Package Insert (document # 200007789)*.

4 Use the Tumor Type field to assign a tumor type for each sample, selecting the most specific tumor type available. Refer to *Select a Tumor Type* on page 6.

5 Use the Tumor Type field to assign one of the following control types for each control. Refer to *Control Samples* on page 6.
- DNA External Control
- RNA External Control
- DNA No-Template Control
- RNA No-Template Control

If using the TruSight Oncology DNA Control, the control type is DNA External Control. If using the TruSight Oncology RNA Control, the control type is RNA External Control.

6 Assign sex.

7 **[Optional]** Select **Export to CSV** to export sample information to an external file.

8 Review the information on the Create Run Screen.

Incorrect information can impact results.

9 Select **Save Run**.

Import Samples

1 Select **Import CSV** and browse to the location of the sample information file. There are two types of files you can import.
   - Select **Download CSV** on the Create Run screen to download a new sample information template. The CSV file contains the required column headings and format for import. Enter sample information in each column for the samples in the run. For the Tumor Type column, enter the tumor type term or associated code (refer to *Download Tumor Types* on page 8). The Tumor Type field is also used to designate samples as controls (refer to *Control Samples* on page 6).
   - Use a file of sample information that was exported from the TSO Comprehensive analysis module using the Export to CSV feature.

2 On the Create Run screen, review the imported information.
Incorrect information can impact results.

3 **[Optional]** Select **Export to CSV** to export sample information to an external file.

4 Select **Save Run**.

Control Samples

TSO Comprehensive assay requires the use of TruSight Oncology Controls. Designating a sample as a control automatically sets the Sex of the sample to Unknown. To designate a sample as a control, select one of four control types from the Tumor Type field: DNA External Control (positive DNA control), DNA No-Template Control, RNA External Control (positive RNA control), or RNA No-Template Control. Refer to *Select a Tumor Type* on page 6 for more information on setting tumor types for all types of samples during run setup.

Only one of each control type may be specified within a run. Only a DNA library may be specified for a DNA External Control or a DNA No-Template Control. Only an RNA library may be specified for an RNA External Control or an RNA No-Template Control. Libraries designated as DNA or RNA No-Template controls are not counted against the maximum number of libraries in a run.

Refer to the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)* for more information on using control samples.

Select a Tumor Type

A tumor type must be specified for each sample. Except for control types, the available tumor types are derived from the installed KB and might change with updated versions of the KB.

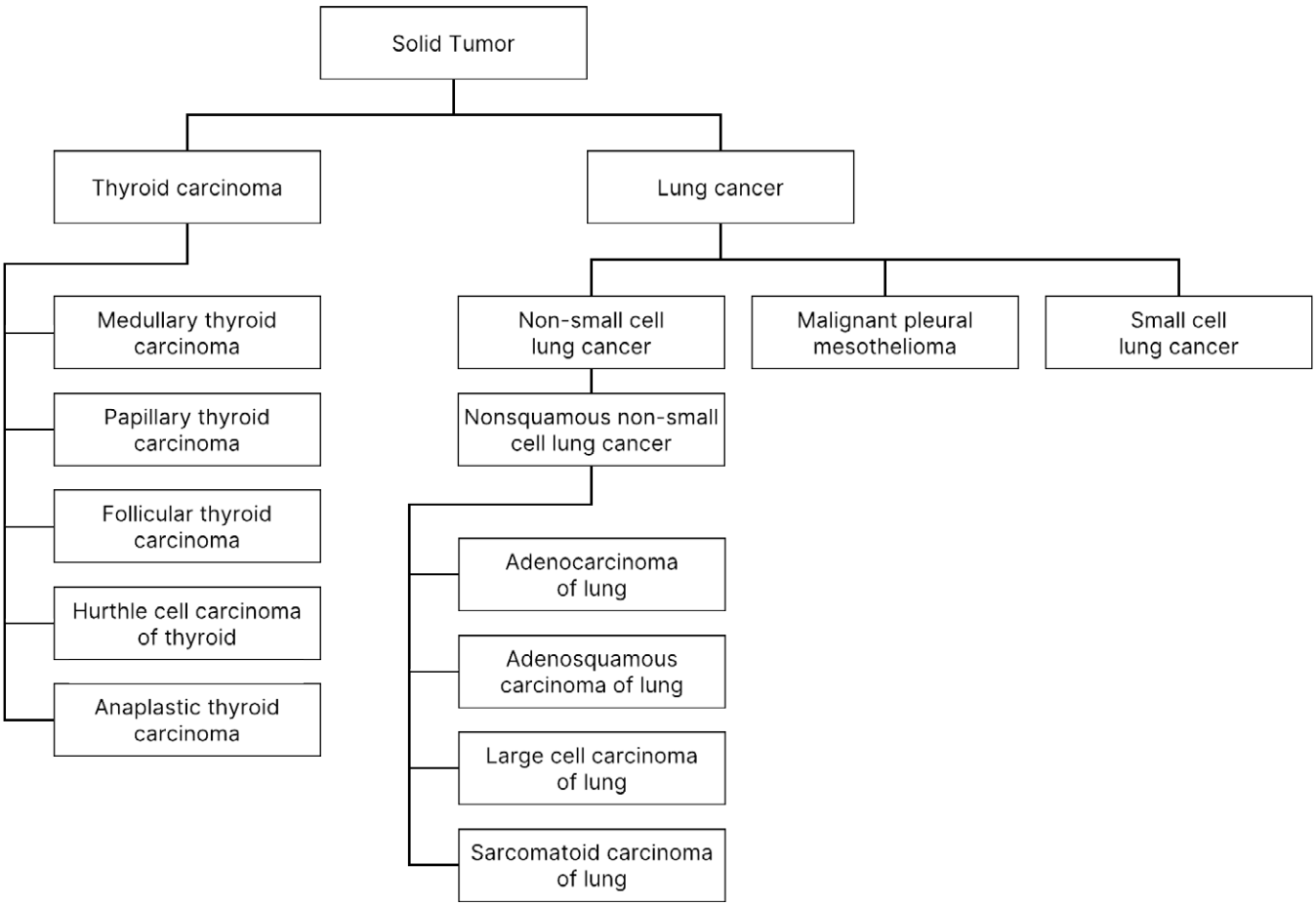
CAUTION

Incorrect selection of tumor type can cause incorrect results. Resolve any warnings that appear when specifying tumor types to avoid analysis failure.

The tumor type terms are part of a hierarchical disease ontology in the KB, which is constructed as a set of parent-child relationships. For example, the term non-small cell lung cancer is a child of lung cancer since non-small cell lung cancer is a type of lung cancer. Figure 1 depicts a subset of an example disease ontology, showing solid tumor as the root term, and the terms associated with lung cancer and thyroid 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, lung cancer is an ancestor of adenocarcinoma of lung and small cell lung cancer, and medullary thyroid carcinoma is a descendant of both thyroid carcinoma and solid tumor.

Figure 1 Subset of an Example Disease Ontology



The selected tumor type for a patient sample impacts:

- Which companion diagnostic intended use(s) are evaluated for the sample. Only patient samples with a tumor type that is an exact match or a descendent of the tumor type for a companion diagnostic intended use will be evaluated for that claim.
- Which tumor profiling variants are included in the TSO Comprehensive assay report. Refer to *Tumor Profiling of Variants* on page 15.

The following instructions describe the process for selecting a tumor type through the Create Run screen. The tumor type can also be set by importing a CSV file containing a tumor type (refer to *Import Samples* on page 6).

1 Display the available tumor types by double-clicking within the Tumor Type cell in the row for the sample. Available tumor types are displayed in a hierarchical list organized alphabetically.
The Tumor Type field is also used to designate a control type for control samples (refer to *Control Samples* on page 6).

2 Locate and select the desired tumor type by interacting with the list or by using the search bar at the top of the Tumor Type window.

Download Tumor Types

A full list of available tumor types in TSV format can be downloaded from the Create Run screen using the **Download Tumor Types TSV** button. The list contains the following information:

- The tumor type term visible in the user interface.
- The full path of the tumor type within the tumor type hierarchy (disease ontology).
- The code used by the TSO Comprehensive analysis module to identify the tumor type.

Edit Run and Initiate Sequencing

For instructions on editing the run information and initiating a sequencing run, refer to the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*. Analysis and Reporting begins once a sequencing run is complete.

For storage considerations, a sequencing run can produce 40–100 GB of output. Secondary analysis of a sequencing run can produce 100–200 GB of output.

Analysis Methods

After sequencing data are collected, they are processed by the TSO Comprehensive analysis module to perform quality control, detect variants, determine Tumor Mutational Burden (TMB) and Microsatellite Instability (MSI) status, determine companion diagnostic results, assess the clinical significance and potential clinical significance of detected variants, and 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, which gives a prediction of the probability of an incorrect base call (Q-score), are also compared to a minimum threshold. If values for each of these three metrics meet the specifications, then Run QC will be reported as PASS and analysis will continue. If a value for any one of the metrics fails to meet the specification, then Run QC will be reported as FAIL and analysis will not proceed. For more information refer to *Quality Control Metrics* on page 41.

FASTQ Generation

Sequencing data stored in BCL format is demultiplexed through a process that uses the index sequences, unique to each sample that was 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), and the combination of those index sequences are used to demultiplex the pooled libraries.

After demultiplexing, this process generates FASTQ files, which 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 involves aligning sequencing reads derived from DNA sample libraries to a reference genome and correcting errors in the sequencing reads prior to variant calling.

The alignment step uses the Burrows-Wheeler Aligner (BWA-MEM) with the SAMtools utility to align DNA sequences in FASTQ files to the hg19 reference genome, generating BAM files (*.bam) and BAM index files (*.bam.bai).

The initial BAM files are further processed to remove errors (including errors introduced during PCR amplification or sequencing), wherein reads derived from the same unique DNA molecule are collapsed into a single representative sequence, leveraging their unique molecular identifier (UMI) incorporated into the library fragments during library preparation.

A second round of alignment using BWA-MEM and SAMtools is performed on the UMI-collapsed reads, resulting in a second set of BAM files with corresponding BAM index files. These BAM files are used as input for gene amplification calling.

Finally, candidate insertions and deletions are identified from the collapsed BAM alignments, and the read pairs are realigned against those candidate insertions and deletions to rescue insertions and deletions signals that may have been missed due to misalignment. Simultaneously, overlapping read pairs are stitched (ie, bioinformatically combined) into a single consensus read. All reads are then output as a third set of BAM files with corresponding BAM index files. These BAM files are used as input for small variant calling, microsatellite instability (MSI) status determination, and DNA library quality control.

Small Variant Calling

Small variant calling is performed for DNA sample libraries (excluding DNA no-template controls) to detect small variants, including single-nucleotide variants (SNVs), multi-nucleotide variants (MNVs) up to 3 base pairs (bp) in length, and insertions and deletions up to 25 bp in length. Certain MNVs, indels (one or more nucleotides replaced by one or more nucleotides and is not an SNV or MNV), and deletions might require a phasing approach to be detected. A predefined set of MNVs, indels, and deletions are detected for the EGFR and RET genes (refer to *Appendix D MNVs, Indels, and Deletions in EGFR and RET Detectable by Phased Variant Caller* on page 46) using a phasing approach. The phasing approach for small variant calling is limited to only these variants. The variant calling algorithms do not differentiate between variants of somatic or germline origin.

Small Variant Detection

The error-corrected BAM files (collapsed and insertions and deletions realigned) are used as input by an initial variant calling algorithm to detect small variants. The initial variant calling step results in unfiltered genome Variant Call Format (gVCF) files, which contain reference or variant case calls for each locus targeted by the TSO Comprehensive assay.

Small Variant Filtering

Candidate variants are then filtered for recurrent (assay-specific) artifacts and formalin-fixed, paraffin-embedded (FFPE) deamination (sample-specific) artifacts. To address assay-specific artifacts, an adjusted quality score is calculated by comparing the observed variant frequency against a baseline noise distribution for the same site. This distribution was derived from profiling a set of normal FFPE samples of varying qualities through the TSO Comprehensive assay. To address sample-specific artifacts, the reads supporting the variant call are stratified by error rate, with reads originating from duplex/stitched reads having the lowest error rate and reads originating from simplex (ie, nonduplex/unstitched) reads having the highest error rate. These error rates are estimated by evaluating all loci with reported variant allele frequencies below 5%. Non-reference reads at these sites are largely due to error, and true somatic events—because of their relative rarity—will not significantly impact these error rate estimates. Because these read classes, duplex/stitched and simplex, have different, sample-specific error rates, confident

detection of a candidate variant may require more or fewer reads as a function of that error rate. For example, at a coverage depth of 200 reads, a variant may be confidently called with three high-quality supporting reads, or with five lower-quality supporting reads.

Candidate variants that do not have sufficient read support based on this error-aware model or which have low adjusted quality scores are tagged with a LowSupport filter flag and are considered as reference calls. In the event that the site also has insufficient coverage for variant calling (less than 100x), the variant is tagged with a LowDP filter flag and is considered as a no-call. Variants with high prevalence in COSMIC3 have lower thresholds for each of these quality metrics compared to non-COSMIC variants. This filtering step results in filtered gVCF files.

Small Variant Phasing

A phased variant caller is used to identify certain MNVs, indels, and deletions in the EGFR and RET genes. The algorithm identifies variants in the EGFR and RET genes that are candidates for phasing in the filtered gVCF files from the previous step and arranges the variants into local neighborhoods. It then mines the error-corrected BAM file for any evidence that these small variants occur in the same clonal sub-populations with each other (ie, in phase with each other). This is done by clustering overlapping reads in the neighborhood into a minimal set of clusters which contain the same variants. Variants are detected by examining the Concise Idiosyncratic Gapped Alignment Report (CIGAR) strings in the BAM file and comparing read sequences to the reference genome sequence.

Small Variant Merging

Finally, MNVs, indels, and deletions detected by the phased variant caller are merged into the filtered gVCF files. Only those MNVs, indels, and deletions from a predefined list of variants in the EGFR and RET genes are eligible for merging into the gVCF (refer to *Appendix D MNVs, Indels, and Deletions in EGFR and RET Detectable by Phased Variant Caller* on page 46). MNVs, indels, and deletions from the phased variant caller take precedence over those that may already exist in the gVCF from the initial variant calling step. This step results in merged gVCF files.

Small Variant Annotation

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

Static Annotation Databases for TMB Calculation

Nirvana is used to annotate filtered small variant calls with static (not updatable) annotation databases for use by downstream TMB calculation (refer to *Tumor Mutational Burden* on page 11). The gVCF from the Small Variant Phasing step (refer to *Small Variant Calling* on page 9) is used as input. Variants detected by the phased variant caller are not used for TMB calculation.

Static Annotation Databases for Companion Diagnostic Calling

Nirvana is used to annotate filtered small variant calls with static (not updatable) annotation databases for use by downstream Companion Diagnostic calling (refer to *Companion Diagnostic Calling* on page 14). The gVCF from the Small Variant Phasing step (refer to *Small Variant Calling* on page 9) is used as input.

Updatable RefSeq Database for Tumor Profiling

Nirvana is used to annotate filtered small variant calls with an updatable RefSeq database as part of a downstream Tumor Profiling of Variants process (refer to *Tumor Profiling of Variants* on page 15). The updatable RefSeq database is included as part of the KB, and may be updated periodically to be compatible with other KB content.

Gene Amplification Calling

Gene amplification calling is performed for DNA sample libraries (excluding DNA no-template controls). An algorithm is used to identify amplified genes and calculate the fold change value for the amplification genes targeted by the TSO Comprehensive assay. A fold change for a given gene is derived from the normalized read depth of the gene in the sample relative to the normalized read depth of diploid regions from the same sample. A fold change exceeding a gene specific cutoff is considered a gene amplification. This analysis step results in a VCF file, summarizing gene amplification status and calculated fold change for each targeted amplification gene

Tumor Mutational Burden

TMB is calculated for DNA sample libraries (excluding DNA no-template controls). A TMB score is generated from the gVCF file generated by the Small Variant Filter step (refer to *Small Variant Calling* on page 9) and the annotations generated during Small Variant Annotations. SNVs and insertions and deletions variants are included in calculating the TMB score, which is derived from the count of non-driver somatic variants per megabase (evaluable region). Driver mutations are identified and filtered based on COSMIC count. While the TSO Comprehensive assay does not differentiate between variants of somatic or germline origin for purposes of small variant calling, variants are flagged as likely germline for the purpose of calculating the TMB score, leveraging a combination of population database and post-database filtering strategies. Variants that are observed frequently across population database are likely of germline origin. After database filtering, the proxi filter labels variants as germline if they are surround by database labeled germline variants. Variants identified as likely germline are excluded from the TMB score calculation. The evaluable region is dynamically adjusted per sample based on sequencing depth. Genomic regions with a high background noise level are excluded from the TMB calculation. TMB is calculated as the number of somatic non-hotspot variants with VAF >=5% divided by the evaluable region size.

Microsatellite Instability Status

To determine the MSI status of a sample, a total of 130 predefined MSI sites are evaluated. For each site, the repeat length distribution is compared against a panel of normal samples to see if the repeat distribution is significantly shifted. The final MSI score is calculated as the number of unstable sites divided by the total number of usable sites (ie, sites with sufficient coverage). A sample is considered MSI-H if its MSI score is >= 20.00%.

Quality Control for DNA Sample Libraries

DNA sample libraries (patient samples only) are assessed for potential contamination by DNA from other samples (foreign DNA) using a combination of a contamination score and a contamination p-value. In contaminated samples, there are germline variants (single nucleotide polymorphisms, or SNPs) that have VAF shifts from expected values of 0%, 50%, or 100%. The algorithm computes a log likelihood score across all common SNP positions where SNV calls are reported. The larger the contamination score, the more likely there is foreign DNA contamination. The rearrangement p-value summarizes a chromosome imbalance score, which represents the overall likelihood of the observed variant calls across each

chromosome. A sample is considered to be contaminated if both the contamination score and rearrangement p-value are above predefined quality thresholds. If contamination is detected, then DNA Library QC will be reported as Fail and no results will be available for small variants, gene amplifications, MSI, or TMB. Additionally, a companion diagnostic or tumor profiling result may not be available if it relies on DNA library QC passing.

QC metrics are used to assess the validity of small variant calling, TMB, MSI, and gene amplifications for DNA sample libraries that pass contamination quality control. If the sample library fails one or more quality metrics, then the corresponding variant type or biomarker is not reported, and the associated QC category in the report header will display as FAIL. Additionally, a companion diagnostic or tumor profiling result may not be available if it relies on QC passing for one or more of the below QC categories.

DNA library QC results are available in the MetricsOutput.tsv file. Refer to *Metrics Output* on page 32.

Low Depth Reporting for DNA Sample Libraries

A Low Depth Report is generated for each patient sample with a DNA library, which 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. Note that it is still possible to detect variants with a total sequencing depth <100 if there is sufficient sequencing depth of the variant allele.

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, and may change with a KB update.

Refer to *Low Depth Report* on page 34 for details on the content.

RNA Alignment

RNA alignment is performed for RNA sample libraries, and includes preprocessing of unaligned sequencing reads, aligning sequencing reads to a reference genome, and postprocessing of aligned sequencing reads.

First, RNA sequences in FASTQ files are downsampled to approximately 30 million reads per RNA sample library. This is done by randomly selecting reads from the input FASTQ files following a probability distribution. Next the ends of RNA sequences are trimmed to a maximum length of 76 base pairs.

Preprocessed reads are then aligned to the hg19 reference genome and candidate splice junctions are identified. This generates BAM files and BAM index files for aligned reads, and a tab-delimited text file for candidate splice junctions.

Finally, duplicate reads are marked in the BAM files, such that they can be excluded from downstream steps. This step generates BAM files and BAM index files that are used as input to RNA Fusion Calling and RNA Splice Variant Calling.

RNA Fusion Calling

Fusion calling is performed for RNA sample libraries (excluding RNA no-template controls). Candidate fusions are identified from anomalous read pairs (ie, reads aligning to different chromosomes or in unexpected orientations) in the BAM files (generated during RNA Alignment) for the fusion genes targeted by the TSO Comprehensive assay. Fusion-supporting reads are assembled into candidate fusion contigs. Candidate fusion contigs are then aligned back to the reference genome. These candidate fusion contigs are then evaluated against a variety of filters prior to being reported as detected. These filters are summarized in the following table.

Filter	Description
Imprecise	A low-resolution candidate, not an assembled fusion call.
RepeatOverlap	The fusion is tagged as overlapping with a repeat region. Only used as a filter for nonuniquely mapping fusion candidates.
WeakBreakend	The read/alignment evidence on one side of the fusion is weak. Usually this filter indicates that the reads only overlap the fusion by a few base pairs. Alternatively, it can indicate too much homology.
DuplicateContig	The two half-contigs of the fusion are comprised of the same sequence.
ContigIntragenic	The realignment of half-contigs produces alignments that map to the same gene on both sides (or within 1 kb if unannotated).
LowQ	Unique fusion supporting reads is less than a predefined threshold (threshold is 5 for 9-16 million reads; 6 for 16-26 million reads; 7 for 26-30 million reads).

Additional fusions may be detected through the RNA Splice Variant Calling process (refer to *RNA Splice Variant Calling* on page 13 and *RNA Fusion Merging* on page 13).

RNA Splice Variant Calling

RNA splice variant calling is performed for RNA sample libraries (excluding RNA no-template controls). Candidate splice variants (junctions) from RNA Alignment are compared against a database of known transcripts and a splice variant baseline of non-tumor junctions generated from a set of normal FFPE samples from different tissue types. Any splice variants that match the database or baseline are filtered out unless they are in a set of junctions with known oncological function. If there is sufficient read support, the candidate splice variant is kept. This process also identifies candidate RNA fusions (refer to *RNA Fusion Merging* on page 13).

RNA Fusion Merging

Fusions identified during RNA Fusion Calling are merged with fusions from proximal genes identified during RNA Splice Variant Calling. These are then annotated with gene symbols or names with respect to a static database of transcripts (GENCODE Release 19). The result of this process is a set of fusion calls that are eligible for reporting.

RNA Splice Variant Annotation

Detected RNA splice variants are annotated using the Nirvana annotation engine with information from the RefSeq database. Annotation of splice variants is performed multiple times independently as described in the following sections.

Static RefSeq Database for Companion Diagnostic Calling

Nirvana is used to annotate detected RNA splice variant calls with a static (not updatable) RefSeq databases for use by downstream Companion Diagnostic calling (see *Companion Diagnostic Calling* on page 14). Splice variants are annotated with transcript-level changes (ie, affected exons in a gene's transcript) with respect to RefSeq. This RefSeq database is the same as the static RefSeq database used by the Small Variant Annotation process.

Updatable RefSeq Database for Tumor Profiling

Nirvana is used to annotate detected RNA splice variant calls with an updatable RefSeq database as part of a downstream Tumor Profiling of Variants process (refer to *Tumor Profiling of Variants* on page 15).

Splice variants are annotated with transcript-level changes (ie, affected exons in a gene's transcript) with respect to RefSeq. The updatable RefSeq database is included as part of the KB, and may be updated periodically to be compatible with other KB content.

Quality Control for RNA Sample Libraries

QC metrics are used to assess the validity of RNA sample libraries. If a QC metric is not within the acceptable range, then RNA Library QC will be reported as FAIL and no results will be available for fusions or splice variants. Additionally, a companion diagnostic or tumor profiling result may not be available if it relies on RNA library QC passing.

RNA library QC results are available in the MetricsOutput.tsv file. Refer to *Metrics Output* on page 32.

Transcripts

A transcript is a strand of RNA that is transcribed from DNA. That RNA can then be translated to create a protein. A gene may have multiple transcripts, such as if different promoters are used, or there are different exon splice patterns. Each transcript has a unique number. In HGVS nomenclature, a nucleotide change that affects a coding sequence can be listed with reference to a transcript, with the first letter indicating the wild type allele and the second letter indicating the variant allele. For example, NM_004333.4:c.1799T>A means that at position 1799 of transcript NM_004333.4, the coding RNA encodes a T in the reference genome, but is changed to an A for this variant.

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 analysis module does not automatically invalidate patient samples based on control sample results.

Refer to the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)* for guidance on run validity and patient sample validity based on results for control samples.

The control output report is available in the ControlOutput.csv file. Refer to *Control Output Report* on page 30.

Companion Diagnostic Calling

For each installed companion diagnostic (CDx) intended use, the TSO Comprehensive analysis module determines the applicability of the CDx intended use for each patient sample based on the patient sample's tumor type. If the patient sample's tumor type 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. See *Select a Tumor Type* on page 6 for more information on the disease ontology. If the patient's tumor type is not applicable to a CDx intended use, then the CDx intended use will not be evaluated for that sample.

If a required sequencing library (DNA or RNA) for a CDx intended use is not sequenced or fails QC, then the patient sample will not be evaluated for that CDx intended use. If a variant type (eg, small variants) or biomarker required for a CDx intended use fails QC, then the patient sample will not be evaluated for that CDx intended use.

Once it is determined 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 will be 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. This is done through an algorithm specific to the CDx intended use, which assess the presence and/or absence of variants/biomarkers that match the CDx intended use.

Companion Diagnostics Results

CDx calling results are made available in the TSO Comprehensive report (see *TruSight Oncology Comprehensive Report* on page 17). Positive CDx intended uses are reported in the Companion Diagnostics Results section of the TSO Comprehensive report.

Tumor Profiling of Variants

After companion diagnostic results are determined, all passing, detected variants in a patient sample are matched against the installed KB to determine the genomic findings that have evidence of clinical significance or have potential clinical significance. This process is called Tumor Profiling of Variants. A genomic finding is either a single variant with evidence of clinical significance or potential clinical significance, or a grouping of variants that, when detected together, have evidence of clinical significance or potential clinical significance.

When multiple variants are listed together as a genomic finding, it means that there is evidence for clinical significance or potential clinical significance for those variants together, in at least one of the sources listed in the Informatics Details of the report. If there are multiple genomic findings, and a variant is included in more than one of these, that variant may be listed more than once on a report. A single variant will only be listed at the highest level where it meets criteria for reporting. Each of the following examples of clinical meaning involved multiple variants:

- NTRK1 p.(Gly595Arg) is indicated to cause resistance to one or more TRK inhibitors, in patients with a qualifying TRK fusion (FDA-approved prescribing information Larotrectinib 211710s000lbl).
- A patient in the LIBRETTO-001 clinical trial was observed to have both RET D898_E901del and RET D903_S904delinsEP. The patient exhibited tumor response to treatment with a RET inhibitor (PMID 32846061).
- An exploratory analysis of the BOLERO-1 and -3 trials suggested that breast cancer patients with ERBB2 amplification derived clinical benefit from mTOR inhibition if the tumors displayed PI3K pathway activation or AKT1 E17K mutations (PMID 27091708).
- A BRAF p.(Val600Glu) mutation co-occurring with TERT promoter mutation is associated with an unfavorable prognosis in papillary thyroid carcinoma per major US guidelines.

Genomic Findings with Evidence of Clinical Significance

Genomic findings with evidence of clinical significance are reported in the Genomic Findings with Evidence of Clinical Significance section of the TSO Comprehensive report (refer to *TruSight Oncology Comprehensive Report* on page 17). Genomic findings are reported in Genomic Findings with Evidence of Clinical Significance if they meet the following criteria:

- The genomic finding is associated with benefit or lack of benefit to a therapy, as evidenced by an EMA-approved drug label or FDA-approved drug label. The sample's tumor type must be equal to or a descendant of the KB association's tumor type in the disease ontology. Refer to *Select a Tumor Type* on page 6 for more information on the disease ontology.
- The genomic finding is associated with benefit or lack of benefit to a therapy, has diagnostic relevance, or has prognostic relevance as evidenced by a published ESMO, ASCO, or other major US clinical practice guideline. The sample's tumor type must be equal to or a descendant of the KB association's tumor type in the disease ontology. Refer to *Select a Tumor Type* on page 6 for more information on the disease ontology

Genomic Findings with Potential Clinical Significance

Genomic findings with potential clinical significance are reported in the Genomic Findings with Potential Clinical Significance section of the TSO Comprehensive report (refer to *TruSight Oncology Comprehensive Report* on page 17). Genomic findings are reported in Genomic Findings with Potential Clinical Significance if they meet the following criteria:

- The genomic finding meets Genomic Findings with Evidence of Clinical Significance criteria (ie, EMA-approved drug label, FDA-approved drug label, ESMO guideline, ASCO guideline, or other major US guideline), but only when the sample's tumor type is not a match to the KB association's tumor type. The sample's tumor type therefore must not be equal to and not be a descendant of the KB association's tumor type.
- The variant has a therapeutic, diagnostic, or prognostic association in the clinical literature describing a clinical study. The sample's tumor type must be equal to or a descendant of the KB association's tumor type.
- The variant is included in eligibility criteria for an enrolling clinical trial (phase I/II, II, II/III, III, or IV) registered at clinicaltrials.gov or the EU Clinical Trials Register (EUCTR). The sample's tumor type must be equal to or a descendant of the clinical trial's tumor type.

TMB and MSI are always reported in Genomic Findings with Potential Clinical Significance, regardless of the sample's tumor type.

Leveling Changes Due to KB Updates

As clinical evidence accumulates for variants in precision oncology, KB updates are made available to reflect the changes. Variants that were initially not reportable due to lack of clinical evidence may later be reported in Genomic Findings with Evidence of Clinical Significance or Genomic Findings with Potential Clinical Significance through a KB content update. Likewise, variants may move from Genomic Findings with Evidence of Clinical Significance to Genomic Findings with Potential Clinical Significance or vice versa. Detected variants not meeting the criteria for any level are not reported. Susceptibility or cancer risk associations are excluded from the KB and do not impact leveling. Therapeutic associations used for leveling are limited to targeted cancer therapies and immunotherapies (not including cell-based immunotherapies).

Positive CDx Results

Companion diagnostic variants reported in Companion Diagnostics Results are excluded from being reported as single-variant genomic findings in Genomic Findings with Evidence of Clinical Significance and Genomic Findings with Potential Clinical Significance. However, genomic findings involving multiple variants might still be reported in Genomic Findings with Evidence of Clinical Significance and Genomic Findings with Potential Clinical Significance, even if one of the variants is reported in Companion Diagnostic Results.

COSMIC Annotations

Variants reported in Genomic Findings with Evidence of Clinical Significance or Genomic Findings with Potential Clinical Significance are annotated with a COSMIC ID, as applicable, from the Catalog of Somatic Mutations in Cancer (COSMIC) database, which is included as part of the KB.

Analysis Output

When the analysis is completed, the Local Run Manager TruSight Oncology Comprehensive Analysis Module generates an analysis folder in the configured output folder for the system. Refer to the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)* for more information on configuring the output folder.

To view analysis output:

1 Navigate to the directory that contains the analysis folder.

2 Open the analysis folder to view output files.
The analysis folder name will be formatted as Analysis_# where # defaults to 1 and is incremented by one for each analysis requeue. A subfolder, YYYYMMDD_HHMMSS, is created inside the analysis folder and indicates the date and time of the analysis (eg, 20210101_145958).

Files

This section describes the summary output files generated during analysis.

Results Reports

TSO Comprehensive reports in PDF and JSON formats are produced for each patient sample that completed analysis successfully. Results are displayed for preview on the Samples and Results tab in the Results Reports section. Samples that did not complete analysis successfully are listed with an error message. Select Export Report to download one TSO Comprehensive report in PDF format. Refer to the analysis output folder for TSO Comprehensive reports for all completed samples.

TruSight Oncology Comprehensive Report

The following tables describe the sections that make up the TSO Comprehensive reports produced for each patient sample in PDF and JSON formats. The PDF report is human readable, while the JSON report is built of 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 N/A for the PDF report. Variants not reported in Companion Diagnostic Results or not meeting the criteria for inclusion in Genomic Findings with Evidence of Clinical Significance or Genomic Findings with Potential Clinical Significance are not included in the reports.

Refer to the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)* for interpretation of results.

Refer to the JSON schema on the TSO Comprehensive support pages on the Illumina support site for additional information on the structure, fields, and possible values in the JSON report.

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

Field in PDF report	Field in JSON report	Description
Report Date	reportDate	Date that the report was generated.
N/A	reportTime	Time that the report was generated.
Sample ID	sampleInformation / sampleId	Sample Identifier. Patient demographics are not included.
Tumor Type	sampleInformation / tumorType	Tumor type associated with the patient sample.

Field in PDF report	Field in JSON report	Description
N/A	sampleInformation / tumorTypeCode	Tumor type code associated with the patient sample.
N/A	sampleInformation / tumorTypePath	Tumor type path (with respect to the disease ontology) associated with the patient sample.
N/A	sampleInformation / tumorTypeCodePath	Tumor type code path (with respect to the disease ontology) associated with the patient sample.
Sex	sampleInformation / sex	Patient sex (Male, Female, or Unknown).
Analysis Date	sampleInformation / analysisDate	Date that the secondary analysis was completed.
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.

- Quality Control—Contains quality control information. For more information on how quality control is evaluated, refer to *Appendix A QC Metrics Flowchart* on page 39.

Field in PDF report	Field in JSON report	Description
Run QC	qualityControl / status / (array item having label = “Run QC”)	Run QC (PASS, FAIL, or N/A) applies to all samples contained in a single sequencing run. PASS—The run is valid. FAIL or N/A—The run is invalid. All RNA and DNA sample-specific QC statuses are N/A (DNA Library QC, DNA MSI QC, DNA Small Variant, and TMB QC, DNA Copy Number Variant QC, RNA Library QC) and there are no variants or biomarkers listed in the report. Refer to the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)* for guidance on run validity and patient sample validity based on results for control samples.
RNA Library QC	qualityControl / status / (array item having label = “RNA Library QC”)	RNA Library QC (PASS, FAIL, or N/A) applies to the RNA library that was sequenced. PASS—the RNA library passed all the RNA-specific QC metrics. FAIL—the RNA library failed one or more of the RNA- specific QC metrics. N/A—the RNA library for the sample was not sequenced, or Run QC had a value of FAIL. If the value is FAIL or N/A, there are no RNA variant types (fusion or splice variants) in the report.
DNA Library QC	qualityControl / status / (array item having label = “DNA Library QC”)	DNA Library QC (PASS, FAIL, or N/A) applies to the DNA library that was sequenced. PASS—the DNA library passed the contamination QC metric. FAIL—the DNA library failed the contamination QC metric. N/A—the DNA library for the sample was not sequenced, or Run QC had a value of FAIL. If the value is FAIL or N/A, no DNA variant types (small variants, copy number variants) or DNA biomarkers (TMB, MSI) are reported.

Field in PDF report	Field in JSON report	Description
DNA MSI QC	qualityControl / status / (array item having label = “DNA MSI QC”)	DNA MSI QC (PASS, FAIL, or N/A) applies to the DNA library that was sequenced. PASS—the DNA library passed the MSI-specific QC metric and upstream DNA Library QC metric. FAIL—the DNA library failed the MSI-specific QC metric. N/A—the DNA library for the sample was not sequenced, DNA Library QC for the sample was FAIL, or Run QC had a value of FAIL. If the value is FAIL or N/A, the biomarker MSI is not reported and listed as Not evaluable.
DNA Small Variant and TMB QC	qualityControl / status / (array item having label = “DNA Small Variant & TMB QC”)	DNA Small Variant and TMB QC (PASS, FAIL, or N/A) apply to the DNA library that was sequenced. PASS—the DNA library passed the Small Variant and TMB specific QC metrics and upstream DNA Library QC metric. FAIL—the DNA library failed one or more of the Small Variant and TMB-specific QC metrics. N/A—the DNA library for the sample was not sequenced, DNA Library QC for the sample was FAIL, or Run QC had a value of FAIL. If the value is FAIL or N/A, there are no small variants in the report, and the biomarker TMB is listed as Not evaluable.
DNA Copy Number Variant QC	qualityControl / status / (array item having label = “DNA Copy Number Variant QC”)	DNA Copy Number Variant (CNV) QC (PASS, FAIL, or N/A) applies to the DNA library that was sequenced. PASS—the DNA library passed all the Copy Number Variant specific QC metrics and upstream DNA Library QC metric. FAIL—the DNA library failed one or more of the Copy Number Variant specific QC metrics. N/A—the DNA library for the sample was not sequenced, DNA Library QC for the sample was FAIL, or Run QC had a value of FAIL. If the value is FAIL or N/A, there are no gene amplifications in the report.

- TruSight Oncology Comprehensive Analysis Module and Knowledge Base Configuration—Contains information on the software and KB versions used when the report was generated.

Field in PDF report	Field in JSON report	Description
Knowledge Base Version	softwareConfiguration / knowledgeBaseVersion	Version of the Knowledge Base installed with the TSO Comprehensive analysis module.
Knowledge Base Published Date	softwareConfiguration / knowledgeBasePublishedDate	Date associated with the Knowledge Base that was used to generate the report.
Module Version	softwareConfiguration / moduleSoftwareVersion	Version of the TSO Comprehensive analysis module used to generate the report.
Claims Package Version	softwareConfiguration / claimsPackageVersion	Version of the Claims Package installed with the TSO Comprehensive 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 which were not evaluated, are listed in the JSON report only. Refer to *Companion Diagnostics Intended Uses Evaluated* on page 23.

Field in PDF report	Field(s) in JSON report	Description
[Message box]	reportFindings / companionDiagnosticResults / 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 either of the following is true for all CDx intended uses: • The sample passes QC, but no associated variant or biomarker was detected or its tumor type is inapplicable. • The sample fails required QC metrics and its tumor type is inapplicable.
[Message box]	reportFindings / companionDiagnosticResults / results / message	A message is optionally displayed in this section. The following message is possible: **One or more biomarkers or variant types failed QC, or the appropriate nucleic acid was not run**—This message is included when at least one CDx intended use applicable to the sample's tumor type could not be evaluated due to a QC failure or due to not having a sequenced DNA or RNA library. Any detected CDx biomarkers appear in a table below this message. Refer to *Companion Diagnostics Intended Uses Evaluated* on page 23 for reasons why a CDx intended use was not evaluated.
N/A	reportFindings / companionDiagnosticResults / results / genomicFindings / (array item for CDx intended use) / companionDiagnosticName	Name of the companion diagnostic intended use. Includes biomarker description, therapy, and tumor type.
Detected Variants/Biomarkers	reportFindings / companionDiagnosticResults / results / genomicFindings / (array item for CDx intended use) / variants	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 / companionDiagnosticResults / results / genomicFindings / (array item for CDx intended use) / therapy	The therapy associated with the CDx intended use.
Usage	reportFindings / companionDiagnosticResults / 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 if the result is not equal to detected. **Indicated**—the associated therapy is indicated for use. **See Note**—a note describes usage of the therapy.
Details	reportFindings / companionDiagnosticResults / results / genomicFindings / (array item for CDx intended use) / note reportFindings / companionDiagnosticResults / results / genomicFindings / (array item for CDx intended use) / variants / (array item for variant in genomic finding)	Contains an optional note and a list of variant details. In the PDF report, the order of variant details corresponds to the order of variants listed for Detected Variants/Biomarkers field. Refer to Table 1, Table 2, Table 3, and Table 4 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.

Field in PDF report	Field(s) in JSON report	Description
N/A	reportFindings / companionDiagnosticResults / 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 sample's tumor type, 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 tumor 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 tumor type. **nucleicAcidNA**—The sample did not have a DNA or RNA 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. **negative**—Placeholder value for future use.

- Other Alterations and Biomarkers Identified—This section contains tumor profiling information for the sample, with detected variants, TMB and MSI categorized into Genomic Findings with Evidence of Clinical significance, or Genomic Findings with Potential Clinical Significance. Refer to *Tumor Profiling of Variants* on page 15 for details on how a level is determined for detected variants.
- Genomic Findings with Evidence of Clinical Significance—Each entry in this section is a genomic finding, which is either a single variant with evidence of clinical significance or a grouping of variants that when detected together have evidence of clinical significance. If no variants are detected, the report displays a No Detected Variants message.

Field in PDF report	Field in JSON report	Description
Detected Variants	reportFindings / otherFindings / genomicFindingsWithEvidenceOfClinicalSignificance / results / genomicFindings / (array item for genomic finding) / variants	A list of detected variants that are part of the genomic finding. For small variants, includes the gene symbol and protein change, transcript change, or genomic change in Human Genome Variation Society (HGVS) format, eg, NRAS p.(Gln61Arg). For gene amplifications, includes the gene symbol followed by Gain, eg, ERBB2 Gain. For fusions, includes the symbols or names of both partner genes (from GENCODE Release 19), separated by a - or /. When separated by a -, the reported gene order corresponds to the transcribed orientation (5' to 3'). When separated by a /, orientation could not be determined. If multiple genes are overlapping a breakpoint, all are listed and delimited by semicolons. For splice variants, includes the gene symbol and affected exon(s) (as applicable), eg, MET Exon 14 skipped.
Details	reportFindings / otherFindings / genomicFindingsWithEvidenceOfClinicalSignificance / results / genomicFindings / (array item for genomic finding) / variants / (array item for variant in genomic finding)	Contains a list of variant details. In the PDF report, the order of variant details corresponds to the order of variants listed for Detected Variants/Biomarkers field. Refer to Table 1, Table 2, Table 3, and Table 4 for a list of variant detail fields.

- **Genomic Findings with Potential Clinical Significance**—TMB and MSI are both reported in this section when there is a sequenced DNA library for the sample. Each other entry in this section is a genomic finding, which is either a single variant with potential clinical significance or a grouping of variants that when detected together have potential clinical significance. If no variants are detected, the report displays a No Detected Variants message.

Field in PDF report	Field in JSON report	Description
TMB	reportFindings / otherFindings / biomarkers / tumorMutationalBurden	TMB is a measurement of the number of estimated somatic mutations carried by tumor cells per megabase in the coding region. TMB is reported as Not evaluable if it could not be evaluated either due to a QC failure or a DNA library for the sample was not sequenced. TMB is always included in Genomic Findings with Potential Clinical Significance.
MSI	reportFindings / otherFindings / biomarkers / microsatelliteInstability	MSI status. Possible values include the following: **MSI-Stable**—Microsatellite stable. **MSI-High**—Microsatellite instability-high. **Not evaluable**—MSI status could not be evaluated either due to a QC failure or a DNA library for the sample was not sequenced. MSI is always included in Genomic Findings with Potential Clinical Significance.
Detected Variants	reportFindings / otherFindings / genomicFindingsWithPotentialClinicalSignificance / results / genomicFindings / (array item for genomic finding) / variants / (all array items) / detectedVariantLabel	A list of detected variants that are part of the genomic finding. For small variants, includes the gene symbol and protein change, transcript change, or genomic change in Human Genome Variation Society (HGVS) format, eg, NRAS p.(Gln61Arg). For gene amplifications, includes the gene symbol followed by Gain, eg, ERBB2 Gain. For fusions, includes the symbols or names of both partner genes (from GENCODE Release 19), separated by a - or /. When separated by a -, the reported gene order corresponds to the transcribed orientation (5' to 3'). When separated by a /, orientation could not be determined. If multiple genes are overlapping a breakpoint, all are listed and delimited by semicolons. For splice variants, includes the gene symbol and affected exon(s) (as applicable), eg, MET Exon 14 skipped.
Details	reportFindings / otherFindings / genomicFindingsWithPotentialClinicalSignificance / results / genomicFindings / (array item for genomic finding) / variants	Contains a list of variant details. In the PDF report, the order of variant details corresponds to the order of variants listed for Detected Variants/Biomarkers field. Refer to Table 1, Table 2, Table 3, and Table 4 for a list of variant detail fields.

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

Field in PDF report	Field in JSON report	Description
[Position list]	reportFindings / companionDiagnosticResults / 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, with a field indicating whether the CDx intended use was evaluated for the sample. If a CDx intended use was not evaluated, a reason is listed.

Field in PDF report	Field in JSON report	Description
Tumor Type	reportFindings / companionDiagnosticResults / qualityControl / intendedUsesEvaluated / companionDiagnosticTable / entries / (array item for CDx intended use) / tumorType	According to the Intended Use statement.
Biomarkers	reportFindings / companionDiagnosticResults / qualityControl / intendedUsesEvaluated / companionDiagnosticTable / entries / (array item for CDx intended use) / biomarkers	According to the Intended Use statement.
Therapy	reportFindings / companionDiagnosticResults / qualityControl / intendedUsesEvaluated / companionDiagnosticTable / entries / (array item for CDx intended use) / therapy	According to the Intended Use statement.
CDx Intended Use Evaluated	reportFindings / companionDiagnosticResults / qualityControl / intendedUsesEvaluated / companionDiagnosticTable / entries / (array item for CDx intended use) / intendedUseEvaluated	Indicates if the CDx intended use was evaluated for the sample (Yes/No). Evaluation of the CDx intended use requires passing the specific QC categories of the nucleic acid or variant/biomarker type associated with the CDx intended use. CDx intended uses associated with detection of small variants (SNV, MNV, Indel) require DNA to be sequenced and the following QC categories to pass: • Run QC • DNA Library QC • DNA Small Variant & TMB QC CDx intended uses associated with the detection of fusions require RNA to be sequenced and the following QC categories to pass: • Run QC • RNA Library QC To be evaluated, the sample's tumor type must either be equal to or a subtype of the tumor type listed in the Companion Diagnostics Intended Uses Evaluated table. Refer to *Select a Tumor Type* on page 6.
Comment	reportFindings / companionDiagnosticResults / qualityControl / intendedUsesEvaluated / companionDiagnosticTable / entries / (array item for CDx intended use) / comment	If CDx Intended Use Evaluated field is Yes and there are no additional comments needed, this field displays a dash. If CDx Intended Use Evaluated field is Yes and there are additional comments to list, a comment such as the following may be displayed. Example: • Some genomic positions associated with the CDx claim had insufficient coverage. Refer to the section Companion Diagnostics Genomic Positions with Insufficient Coverage for Small Variant Detection for details. If CDx Intended Use Evaluated field is No, a comment such as the following is displayed. Examples: • Tumor Type of sample does not match tumor type corresponding to the CDx Intended Use. • DNA or RNA data associated with a CDx biomarker. not available • Required QC category did not pass.

- **About the Test, Informatics Details, Limitations**—Contains general information about the test as well as a list of limitations.

Field in PDF report	Field in JSON report	Description
About the Test	about / description	Test description.
Informatics Details	details / (one JSON property per subsection)	A brief description of the report sections and other informatics details.
Limitations	limitations / description	List of assay and report limitations.

- **TruSight Oncology Comprehensive Gene Panel**—Contains information about the gene panel.

Field in PDF report	Field in JSON report	Description
Gene Panel	genePanel / geneList / genes genePanel / geneList / genes / variants	The list of genes that are part of the panel, including a footnote indicating which variant types are evaluated for which genes. Small variants are called in all genes.

Table 1 Small Variant Details in Report

Field in PDF report	Field in JSON report (relative path in variant JSON object)	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 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 (i.e., 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.
Alternate Allele	additionalInfo / (array item having label property = "Alternate Allele") / value	Alternate allele.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
N/A	cosmicIds	List of genomic mutation IDs associated with the variant from the Catalogue of Somatic Mutations In Cancer (COSMIC) database, as applicable.
N/A	detailedSmallVariantData / vcfChromosome	Chromosome.
N/A	detailedSmallVariantData / vcfPosition	Genomic position (hg19). Refers to the position of the first base in the reference allele (detailedSmallVariantData / referenceAllele field).
N/A	detailedSmallVariantData / vcfRefAllele	The reference allele.
N/A	detailedSmallVariantData / vcfVariantFrequency	Variant allele frequency.
N/A	detailedSmallVariantData / annotation / transcripts	Detailed transcript-level annotations for a transcript (as applicable). Only a single preferred transcript is included.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / transcript	Transcript ID.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / source	Transcript source (eg, 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 (eg, 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 (eg, 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 would indicate that exons 4, 5, and 6 were affected and that this transcript contains 7 exons in total.
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.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
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 (ie, 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.
N/A	detailedSmallVariantData / 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	detailedSmallVariantData / annotation / transcripts / (first array item) / proteinId	Protein ID.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / proteinPos	Protein position.

Annotations (positional information, consequences, etc.) provided in Table 1 are based on variants that have been left-aligned to the genome in accordance with next-generation sequencing norms. The one exception to this rule is that HGVS notation is right-aligned with the respective reference sequence according to the HGVS standard. When insertions and deletions occur in low complexity genomic regions, the left-aligned and right-aligned representations might refer to different locations.

Table 2 Gene Amplification Details in Report

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
Type	type / value	The detailed type of variant. Possible values for gene amplifications include: **CNV**—Copy number variant (gene amplifications are the only copy number variants listed in the report).
Fold Change	detailedCopyNumberVariantData / foldChange	The fold-change of normalized read depth in the sample relative to the normalized read depth in diploid genomes.
N/A	detailedCopyNumberVariantData / copyNumberType	Value is <DUP> for all gene amplifications.
N/A	detailedCopyNumberVariantData / gene	Gene symbol.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
N/A	detailedCopyNumberVariantData / chromosome	Chromosome of the gene.
N/A	detailedCopyNumberVariantData / startPosition	Start position (hg19) of the gene.
N/A	detailedCopyNumberVariantData / endPosition	End position (hg19) of the gene.

Table 3 Fusion Details in Report

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
Type	type / value	The detailed type of variant. Possible values for fusions include: Fusion
Breakpoint 1	additionalInfo / (array item having label property = “Breakpoint 1”) / value	Observed fusion breakpoint 1 in RNA. Chromsome:position format (hg19).
Breakpoint 2	additionalInfo / (array item having label property = “Breakpoint 2”) / value	Observed fusion breakpoint 2 in RNA. Chromsome:position format (hg19).
Fusion Supporting Reads	additionalInfo / (array item having label property = “Fusion Supporting Reads”) / value	Count of fusion supporting reads.
N/A	detailedGeneFusionData / fusionDirectionalityKnownAndIndiciatedByGeneOrder	Displays true when gene/breakpoint order corresponds to the transcribed orientation (5’ to 3’). Displays false when orientation could not be determined.
N/A	detailedGeneFusionData / fusionSupportingReads	Count of fusion supporting reads.
N/A	detailedGeneFusionData / partner1 / gene	Symbols or name (from GENCODE Release 19) of gene(s) overlapping Breakpoint 1. Multiple genes overlapping the same breakpoint are delimited by semicolons.
N/A	detailedGeneFusionData / partner1 / chromosome	Chromosome of breakpoint 1.
N/A	detailedGeneFusionData / partner1 / position	Position (hg19) of breakpoint 1.
N/A	detailedGeneFusionData / partner2 / gene	Symbols or name (from GENCODE Release 19) of gene(s) overlapping Breakpoint 2. Multiple genes overlapping the same breakpoint are delimited by semicolons.
N/A	detailedGeneFusionData / partner1 / chromosome	Chromosome of breakpoint 2.
N/A	detailedGeneFusionData / partner1 / position	Position (hg19) of breakpoint 2.

Table 4 Splice Variant Details in Report

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
Type	type / value	The detailed type of variant. Possible values for splice variants include: Splice Variant
Affected Exon(s)	additionalInfo / (array item having label property = “Affected Exon(s)”) / value	The exon(s) affected by the splice variant, as applicable. For examples, 4-6 would indicate that exons 4, 5, and 6 were affected.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
Transcript	additionalInfo / (array item having label property = “Transcript”) / value	Transcript ID (RefSeq).
Breakpoint Start	additionalInfo / (array item having label property = “Breakpoint Start”) / value	Observed splice variant breakpoint start in RNA. Chromsome:position format (hg19).
Breakpoint End	additionalInfo / (array item having label property = “Breakpoint End”) / value	Observed splice variant breakpoint end in RNA. Chromsome:position format (hg19).
Splice Supporting Reads	additionalInfo / (array item having label property = “Splice Supporting Reads”) / value	Count of splice supporting reads.
N/A	detailedSpliceVariantData / breakPointStartChromosome	Chromosome of breakpoint start.
N/A	detailedSpliceVariantData / breakPointStartPosition	Position (hg19) of breakpoint start.
N/A	detailedSpliceVariantData / breakPointEndChromosome	Chromosome of breakpoint end.
N/A	detailedSpliceVariantData / breakPointEndPosition	Position (hg19) of breakpoint end.
N/A	detailedSpliceVariantData / spliceSupportingReads	Count of splice supporting reads.
N/A	detailedSpliceVariantData / annotation / source	Transcript source (e.g., RefSeq).
N/A	detailedSpliceVariantData / annotation / gene	Gene symbol.
N/A	detailedSpliceVariantData / annotation / affectedExons	The exon(s) affected by the splice variant, and total exon count, as applicable. For examples, 4-6/7 would indicate that exons 4, 5, and 6 were affected and that this transcript contains 7 exons in total.
N/A	detailedSpliceVariantData / annotation / transcript	Transcript ID.

Sample Sheet

File name: SampleSheet.csv

For each analysis, the TSO Comprehensive analysis module creates a comma-delimited sample sheet (SampleSheet.csv). This file contains sample information provided to the software during the run setup. These sample sheets contain a header with information about the run and descriptors for the sample libraries processed in a particular flow cell (one data row per sample library).

CAUTION

Modifying the sample sheet file will cause adverse effects downstream, including incorrect results or analysis failure.

The following table provides sample sheet data details:

Column Name	Description
Sample_ID	Sample ID with "-DNA" appended for DNA libraries or "-RNA" appended for RNA libraries.
I7_Index_ID	i7 index name. Refer to *Illumina Adapter Sequences (document # 1000000002694)* for details on how the sample sheet index ID maps to the index ID entered during run setup.
index	i7 index sequence.
I5_Index_ID	i5 index name. Refer to *Illumina Adapter Sequences (document # 1000000002694)* for details on how the sample sheet index ID maps to the index ID entered during run setup.
index2	i5 index sequence.
Sample_Type	DNA or RNA.
Pair_ID	Sample ID (same ID is used for a DNA library and RNA library from the same sample).
Sample_Description	Sample description.
Tumor_Type	Tumor type for patient samples. Control type for control samples.
Sex	Sex (Male, Female or Unknown).

Control Output Report

File name: ControlOutput.csv

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 analysis module does not automatically invalidate patient samples based on control sample results. Refer to the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)* for guidance on run validity and patient sample validity based on results for control samples.

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

- Control Types—Contains information about each control sample included in the run.

Field	Description
Control Type	The control type of the control sample. Possible values include DNA External Control, DNA No-Template Control, RNA External Control, or RNA 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, NA.
Overall Result	The QC result for the control sample. Possible values include PASS, FAIL, NA.
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 the following control types: DNA External Control and RNA External Control.
Sensitivity Threshold	The minimum sensitivity value required for the control sample to have a QC result of PASS. Only applicable for the following control types: DNA External Control and RNA External Control.

- 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 analysis module.
Pipeline Version	The version of the analysis pipeline/workflow.

- 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 Control Software (NCS) 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 steps of the analysis. Possible values include TRUE, FALSE, NA. If the value is FALSE, contact Illumina technical support for more information.
FAILED_STEPS	A list of any failed analysis steps due to a software error. Contact Illumina technical support for more information if any step is listed here.
STEPS_NOT_ EXECUTED	A list of any analysis steps not executed due to a software error. Contact Illumina technical support for more information if any step is listed here.

- Small Variants Truth Table Results—Contains information on which control DNA small variants in the DNA External Control (positive DNA control) were detected or not detected (one row per control variant). NA values will be listed if the DNA External Control was not 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, NA.
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.

- Splice Variants Truth Table Results—Contains information on which control RNA splice variants in the RNA External Control (positive RNA control) were detected or not detected (one row per control variant). NA values will be listed if the RNA External Control was not included in the sequencing run.

Field	Description
Detected	Indicates whether the control RNA splice variant was detected in the control sample. Possible values include TRUE, FALSE, NA.
HGNC Gene Name	HGNC gene symbol associated with the control RNA splice variant.
Breakpoint 1	Chromosome and position (hg19) of the first breakpoint of the control RNA splice variant.
Breakpoint 2	Chromosome and position (hg19) of the second breakpoint of the control RNA splice variant.

- 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). NA values will be listed if the RNA External Control was not included in the sequencing run.

Field	Description
Detected	Indicates whether the control RNA fusion variant was detected in the control sample. Possible values include TRUE, FALSE, NA.
HGNC Gene Name 1	HGNC gene symbol associated with the first breakpoint of the control RNA fusion variant.
HGNC Gene Name 2	HGNC gene symbol associated with the second breakpoint of the control RNA fusion variant.

- 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. NA values will be 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

- RNA NTC Library QC Metrics—Contains information on the quality control metric that was evaluated for the RNA 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. NA values will be listed if the RNA No-Template Control was not included in the sequencing run.

Metric	Description	Units	Quality Threshold
GENE_ABOVE_ MEDIAN_CUTOFF	The number of genes for which the median deduped read depth across all loci spanned for each gene is > 20.	Count	≤ 1

Metrics Output

File name: MetricsOutput.tsv

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

The metrics output file contains the following sections and their 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 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 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 8 for analysis details. Refer to *Quality Control Metrics* on page 41 for metric descriptions and thresholds.

Column	Description
Metric (UOM)	QC metric name and unit of measurement.
LSL	Lower specification limit (inclusive).
USL	Upper specification limit (inclusive).
Value	QC metric value.
PASS/FAIL	Indicates whether the sample passed or failed the quality control metric. Possible values include PASS, FAIL or NA.

- Analysis Status—Contains information on whether analysis completed for each patient sample, and whether any samples failed due to a software error. Each column in this section corresponds to a patient sample (Sample ID is used for the column name).

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, contact Illumina technical support for more information.
FAILED_STEPS	A list of any failed analysis steps due to a software error. Contact Illumina technical support for more information if any step is listed here.
STEPS_NOT_EXECUTED	A list of any analysis steps not executed due to a software error. Contact Illumina technical support for more information if any step is listed here.

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

Section	Description	Corresponding QC Category in TSO Comprehensive Report
DNA Library QC Metrics	QC metrics used as validity criteria for DNA sample libraries. Refer to *Quality Control for DNA Sample Libraries* on page 11 for analysis details. Refer to *Quality Control Metrics* on page 41 for metric descriptions and thresholds.	DNA Library QC
DNA Library QC Metrics for Small Variant Calling and TMB	QC metrics used as validity criteria for small variants and TMB in a DNA sample library. Refer to *Quality Control for DNA Sample Libraries* on page 11 for analysis details. Refer to *Quality Control Metrics* on page 41 for metric descriptions and thresholds.	DNA Small Variant & TMB QC
DNA Library QC Metrics for MSI	QC metrics used as validity criteria for MSI in a DNA sample library. Refer to *Quality Control for DNA Sample Libraries* on page 11 for analysis details. Refer to *Quality Control Metrics* on page 41 for metric descriptions and thresholds.	DNA MSI QC
DNA Library QC Metrics for CNV	QC metrics used as validity criteria for gene amplifications in a DNA sample library. Refer to *Quality Control for DNA Sample Libraries* on page 11 for analysis details. Refer to *Quality Control Metrics* on page 41 for metric descriptions and thresholds.	DNA Copy Number Variant 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 11 for analysis details. Refer to *DNA Expanded Metrics* on page 42 for metric descriptions.	N/A
RNA Library QC Metrics	QC metrics used as validity criteria for RNA sample libraries. Refer to *Quality Control for RNA Sample Libraries* on page 14 for analysis details. Refer to *Quality Control Metrics* on page 41 for metric descriptions and thresholds.	RNA Library QC
RNA Expanded Metrics	RNA Expanded Metrics are for information only and do not directly indicate the quality of RNA libraries. Refer to *Quality Control for RNA Sample Libraries* on page 14 for analysis details. Refer to *RNA Expanded Metrics* on page 43 for metric descriptions and thresholds.	N/A

Each section contains the following columns:

- Metric (UOM)—The QC metric name and unit of measurement.
- LSL—Lower specification limit (inclusive).
- USL—Upper specification limit (inclusive).

- One column per sample (named with Sample ID).

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 that 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 their associated fields:

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

Field	Description
Sample ID	Sample ID of the patient sample.
Tumor Type	Tumor type of the patient sample.
Report Date	The date the low depth report was generated.
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.
LRM Module version	The version of the TSO Comprehensive analysis module.

- 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.

Output Folder Structure

This section describes the content of each output folder generated during analysis.

- IVD
  - IVD_Reports
    - {SampleID}_TSOCompEUModule_KB{version}_Report.pdf—TSO Comprehensive report (PDF format) per patient sample

- {SampleID}_TSOCompEUModule_KB{version}_Report.json—TSO Comprehensive report (JSON format) per patient sample
- {SampleID}_LowDepthReport.tsv—Low depth report per patient sample
- MetricsOutput.tsv—Metrics output
- ControlOutput.tsv—Control output report

- Logs_Intermediates—Logs and intermediate files generated during the analysis pipeline/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/pipeline. The TSO Comprehensive analysis module appends RNA or DNA to the Sample ID folder names during processing.

View Analysis Results

1 From the Local Run Manager dashboard, select the run name.

2 From the Run Overview tab, review the sequencing run metrics.

3 To change the analysis data file location for future requeues of the selected run, select **Edit**, and then edit the output run folder file path.
The output run folder name cannot be changed.

4 **[Optional]** Select **Copy to Clipboard** for access to the output run folder.

5 Select the Sequencing Information tab to review run parameters and consumables information.

6 Select the Samples & Results tab to view reports and quality control information.
- If analysis was repeated, expand the Select Analysis drop-down list and select the appropriate analysis.

7 **[Optional]** Select **Copy to Clipboard** to copy the Analysis folder file path.

For more information on the Run Overview and Sequencing Information tabs, and how to requeue analysis, refer to *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Samples & Results

The Samples & Results screen displays the analysis results associated with the selected run and provides the option to reanalyze the run with different parameters. A table at the top of the screen provides the start date of the currently selected analysis run and the type of run (initial analysis, analysis requeue, or report regeneration).

Run Level Metrics

The *Run Level Metrics* section of the Samples & Results screen displays a run QC metric status of PASS or FAIL for each Run QC metric. Run QC metric statuses are sourced from the MetricsReport.tsv file (refer to *Metrics Output* on page 32). Refer to *Quality Control Metrics* on page 41 for metric descriptions and thresholds.

Control Samples

Control samples are designated in the Run Setup screen of Local Run Manager. Results for samples designated as controls are displayed in the *Controls* section of the Samples & Results screen. The Controls section displays the following columns for each sample designated as a control:

- Sample ID
- Type— Control sample type. Possible values are DNA External Control, DNA No-Template Control, RNA External Control, and RNA No-Template Control. Available control sample types remain the same and are not affected by the knowledge base installed.
- Analysis Complete?— Possible values are TRUE and FALSE. Control samples marked as TRUE in the Analysis Complete? column have completed control sample analysis. If a control sample is marked FALSE, a software error has occurred. Contact Illumina technical support for more information.
- Outcome—Possible values are PASS and FAIL. Refer to the following table for outcome value interpretation:

Control sample type	Outcome	Interpretation
DNA No-Template	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.
RNA No-Template	PASS	Cross-contamination between libraries is not indicated.
	FAIL	Cross-contamination between libraries is indicated. RNA samples in the library preparation event and all associated sequencing runs are invalid.
DNA External	PASS	Expected variants have been detected.
	FAIL	Variant calling specifications have not been met and DNA samples in the sequencing run are invalid.
RNA External	PASS	Expected variants have been detected.
	FAIL	Variant calling specifications have not been met and RNA samples in the sequencing run are invalid.

Sample Level Metrics

The Sample Level Metrics section of the Samples & Results screen displays quality control information for patient samples that were included in the run. Patient sample quality control results are sourced from the MetricsReport.tsv file (refer to *Metrics Output* on page 32). The Sample Level Metrics section displays the following columns for each patient sample:

- Sample—The sample ID.
- Analysis Complete?—Possible values are TRUE and FALSE. Samples marked as TRUE in the Analysis Complete? column have completed analysis successfully. If a sample is marked FALSE in this column, a software error has occurred. Contact Illumina technical support for more information.
- DNA Library QC—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 report. A dash (–) is shown if a DNA library was not sequenced, or Run QC has a value of FAIL.
- DNA Variants and Biomarkers
  - 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 report. A dash (–) 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
  - MSI—Possible value are PASS and FAIL. Indicates whether the sample passed or failed QC for MSI in the DNA library. Corresponds to DNA MSI QC in the TSO Comprehensive report. A dash (–) 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

- CNV—Possible value are PASS and FAIL. Indicates whether the sample passed or failed QC for gene amplifications in the DNA library. Corresponds to DNA Copy Number Variant QC in the TSO Comprehensive report. A dash (–) 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.

- RNA Library QC—Possible values are PASS and FAIL. Indicates whether the sample passed or failed RNA library QC, which applies to the RNA library that was sequenced. Corresponds to RNA Library QC in the TSO Comprehensive report. A dash (–) is shown if an RNA library was not sequenced, or Run QC has a value of FAIL.

Individual samples can fail, even when run metrics pass.

Report Regeneration

Report regeneration allows one or more reports to be regenerated without repeating all secondary analysis steps. Report regeneration is much faster than a full analysis requeue but has different features:

- Scope —Report regeneration rebuilds the TSO Comprehensive report but skips some analysis steps. You can change the sex or tumor type for one or more samples or install a new KB to produce a new report reflecting these changes. Each sample must be manually selected for report regeneration, while an analysis requeue automatically selects all samples by default. Individual samples can be removed for analysis requeue.
- Analysis run failure—Report regeneration requires a successful analysis run as input, while analysis requeue can be used in scenarios where analysis has failed.
- Editable fields—Report regeneration allows changes to the Sex and Tumor Type fields, while analysis requeue allows any of the fields selected during run setup to be changed.
- TSO Comprehensive analysis module version—Report regeneration requires a successful analysis from Local Run Manager TruSight Oncology Comprehensive Analysis Module v2.3 or later. An analysis requeue can be initiated using analysis from any previous version of TSO Comprehensive analysis module.
- Run Input Settings—Report regeneration run inputs are automatically set to the values from the most recent successful secondary analysis run. The run inputs for an analysis requeue are automatically set to the values from the most recent analysis attempt (including failed analysis runs).

This feature is only accessible to LRM admin users or a non-admin user with requeue analysis permissions assigned. For more information on LRM user management, refer to *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Regenerate a Report or Requeue Analysis

1. From the run dashboard, locate a run with a status of Analysis Completed. Select the vertical ellipses icon and select Requeue.
   Relinking runs that have been deleted from the local temp folder is required to requeue analysis. For more information on LRM user management, refer to *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.
2. Select Edit Setup in the Requeue Analysis pop-up.
3. Use the dropdown at the top of the Requeue Analysis screen to select report regeneration or full analysis requeue.

NOTE Always review run inputs for each sample before saving a run. Report regeneration run inputs are automatically set to the values from the most recent successful secondary analysis run.

4 Samples from the previously completed run will be displayed in a table. Use the + buttons on the right of the table to mark desired samples for report regeneration. All samples in a run are excluded from report regeneration by default and must be added individually. Report regeneration is not available for samples originally analyzed as control samples, which require full analysis requeue.

5 When all desired samples have been marked for report regeneration, select **Requeue Analysis**.

Viewing Report Regeneration Results

Regenerated reports for samples marked for report regeneration can be viewed along with other completed analyses in the Samples and Runs screen in Local Run Manager. Reports produced using report regeneration are marked as Report Regeneration in the Analysis Type field at the top of the Samples and Runs screen.

Troubleshooting

When the sample report indicates that the analysis for the sample failed due to a software error, troubleshoot the issue based on the specific failed step. In the IVD_Reports folder, the MetricsOutput.tsv indicates the specific analysis step that did not complete under FAILED_STEPS.

Use the following table to troubleshoot issues in the workflow.

Failed Step	Recommended Action
FastqValidation	If the software error is due to the FastqValidation step, then one possible cause is an incorrect or nonexistent index resulting in no reads for the sample. If an incorrect index is suspected, then analysis should be repeated with the correct index identifier selected. Otherwise, the sample should be repeated through the TSO Comprehensive workflow with a new extraction of nucleic acid in accordance with the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)*.
FusionCalling	If the software error is due to the FusionCalling step, then the possible causes are a poor-quality sample (insufficient intact RNA), insufficient input of RNA, a use error during the TSO Comprehensive workflow, or an incorrect index assigned to the sample. The sample should be repeated through the TSO Comprehensive workflow with a new extraction of nucleic acid in accordance with the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)*.

For any other steps that are indicated as failed, contact Illumina Technical Support.

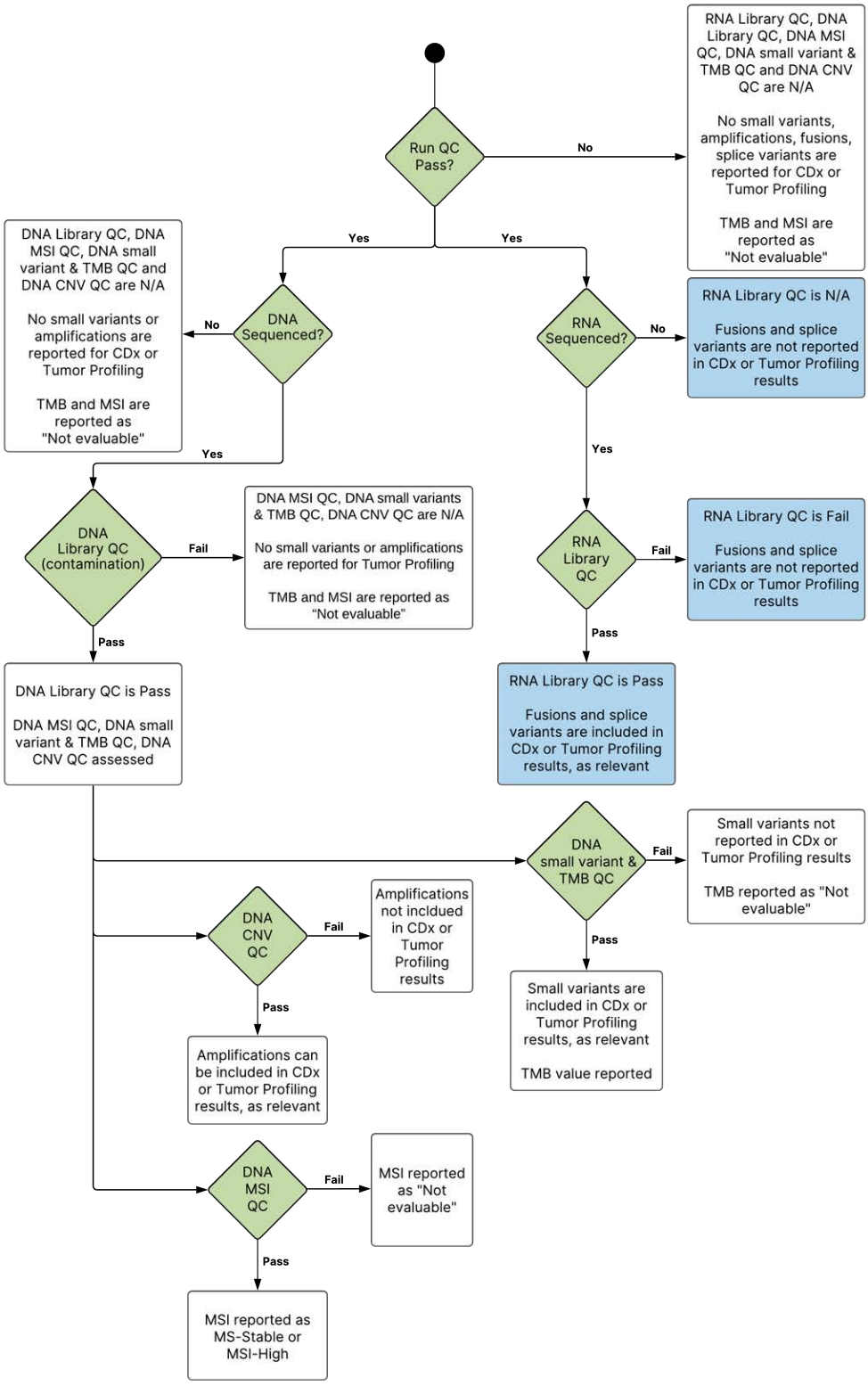
Appendix A QC Metrics Flowchart

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

- *Analysis Methods* on page 8
- Quality Control table on page 18
- Run QC Metrics table on page 32
- *Quality Control for DNA Sample Libraries* on page 11
- *Sample Level Metrics* on page 36
- *Appendix B QC Metrics* on page 41

The flowchart does not map the control samples. The results from the control samples do not impact the QC metrics on the TSO Comprehensive PDF or JSON report. The use of control samples is described in *Control Samples* on page 6. For additional control samples information, refer to the *TruSight Oncology Comprehensive (EU) Package Insert (document # 200007789)*.

The flowchart does not map the position-level QC results. These results are part of the Companion Diagnostic QC results, which are described in the Companion Diagnostic QC table on page 22. Position-level QC results for the Tumor Profiling section are provided in the Low Depth Report, which is described in *Low Depth Reporting for DNA Sample Libraries* on page 12.


Run QC Pass?
No
RNA Library QC, DNA Library QC, DNA MSI QC, DNA small variant & TMB QC and DNA CNV QC are N/A
No small variants, amplifications, fusions, splice variants are reported for CDx or Tumor Profiling
TMB and MSI are reported as "Not evaluable"
Yes
Yes
DNA Sequenced?
No
DNA Library QC, DNA MSI QC, DNA small variant & TMB QC and DNA CNV QC are N/A
No small variants or amplifications are reported for CDx or Tumor Profiling
TMB and MSI are reported as "Not evaluable"
RNA Sequenced?
No
RNA Library QC is N/A
Fusions and splice variants are not reported in CDx or Tumor Profiling results
Yes
Yes
DNA Library QC (contamination)
Fail
DNA MSI QC, DNA small variants & TMB QC, DNA CNV QC are N/A
No small variants or amplifications are reported for Tumor Profiling
TMB and MSI are reported as "Not evaluable"
RNA Library QC
Fail
RNA Library QC is Fail
Fusions and splice variants are not reported in CDx or Tumor Profiling results
Pass
Pass
DNA Library QC is Pass
DNA MSI QC, DNA small variant & TMB QC, DNA CNV QC assessed
RNA Library QC is Pass
Fusions and splice variants are included in CDx or Tumor Profiling results, as relevant
DNA small variant & TMB QC
Fail
Small variants not reported in CDx or Tumor Profiling results
TMB reported as "Not evaluable"
DNA CNV QC
Fail
Amplifications not incldued in CDx or Tumor Profiling results
Pass
Pass
Small variants are included in CDx or Tumor Profiling results, as relevant
TMB value reported
Amplifications can be included in CDx or Tumor Profiling results, as relevant
DNA MSI QC
Fail
MSI reported as "Not evaluable"
Pass
MSI reported as MS-Stable or MSI-High

Appendix B QC Metrics

Quality Control Metrics

Table 5 TSO Comprehensive Report Result QC Metrics

Output Type	Metric	Specification	Description	Impact of Specification Failure*
Sequencing Run	PCT_PF_READS (%)	≥ 80.0	Percentage of reads passing filter (PF).	Sequencing run invalidated, no results reported for any sample in the run.
	PCT_Q30_R1 (%)	≥ 80.0	Average percent of base calls with quality score of Q30 or higher for Read 1.	
	PCT_Q30_R2 (%)	≥ 80.0	Average percent of base calls with quality score of Q30 or higher for Read 2.	
DNA Libraries	CONTAMINATION_SCORE	≤ 3106 OR > 3106 and P_VALUE ≤ 0.049	A metric assessing the likelihood of contamination using the VAF of common variants. The contamination score is based on VAF distribution of SNPs. The contamination P value used to assess highly rearranged genomes, only applicable when contamination score is above Upper Spec Limit.	No DNA results reported.
	MEDIAN_INSERT_SIZE (bp)	≥ 70	The median fragment length in the sample.	No TMB or small DNA variant results reported.
	MEDIAN_EXON_COVERAGE (count)	≥ 150	Median exon fragment coverage across all exon bases.	
	PCT_EXON_50X (%)	≥ 90.0	Percent exon bases with 50X fragment coverage.	
	USABLE_MSI_SITES (count)	≥ 40	The number of MSI sites usable for MSI calling (Number of microsatellite sites with sufficient spanning reads to identify microsatellite instability).	No MSI results reported.
	COVERAGE_MAD (count)	≤ 0.210	The median of absolute deviations from the median of the normalized count of each CNV target region.	No gene amplification results reported.
	MEDIAN_BIN_COUNT_CNV_TARGET (count)	≥ 1.0	The median raw bin count per CNV target.	

Output Type	Metric	Specification	Description	Impact of Specification Failure*
RNA Libraries	MEDIAN_INSERT_SIZE (bp)	≥ 80	The median fragment length in the sample.	No fusions or splice variant results reported.
	MEDIAN_CV_GENE_500X (coefficient)	≤ 0.93	MEDIAN_CV_GENE_500X is a measure of coverage uniformity. For each gene with at least 500x coverage, the coefficient of variation in coverage across the gene body is computed. This metric is the median of these values. A high value indicates a high level of variation and indicates a problem in library preparation such as low sample input and/or probe pulldown issues. This metric is computed using all reads (including reads marked as duplicates).	
	TOTAL_ON_TARGET_READS (count)	≥ 9,000,000	The total number of reads that map to the target regions. This metric is computed using all reads (including reads marked as duplicates).	

*Successful results show PASS.

DNA Expanded Metrics

DNA expanded metrics are provided for information only. They 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
PCT_CHIMERIC_READS	Percent of chimeric reads	%
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	%
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.	%

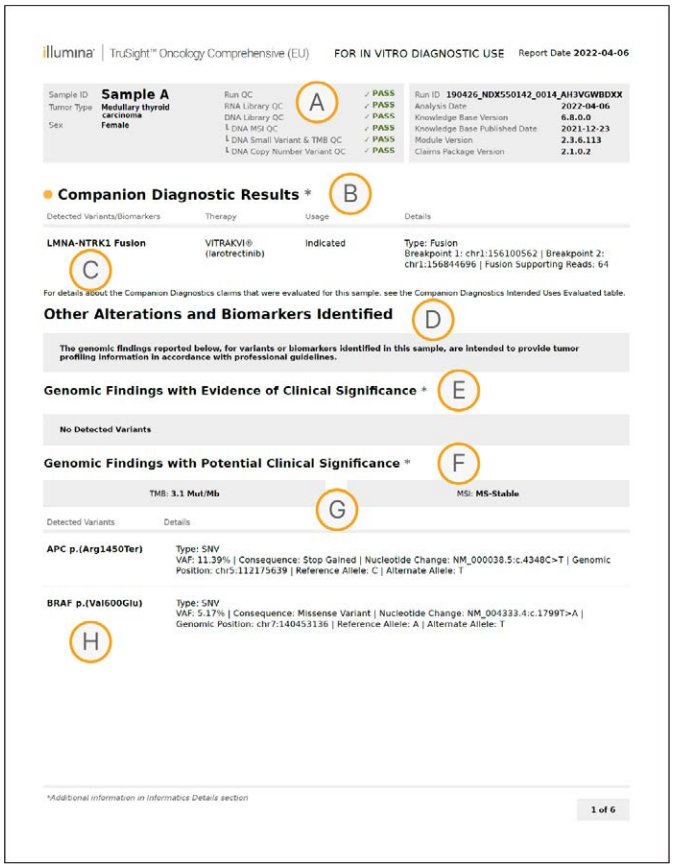
Metric	Description	Units
PCT_ CONTAMINATION_ EST	Percent of contamination of the sample	%
PCT_PF_UQ_ READS	Percent unique reads passing filter	%
PCT_TARGET_ 0.4X_MEAN	Percent target bases with target coverage greater than .4 times the mean	%
PCT_TARGET_100X	Percent target bases with greater than 100X coverage	%
PCT_TARGET_250X	Percent target bases with greater than 250X coverage	%

RNA Expanded Metrics

RNA expanded metrics are provided for information only. They 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
PCT_CHIMERIC_ READS	Percentage of reads that are aligned as two segments which map to non-consecutive regions in the genome	%
PCT_ON_TARGET_ READS	Percentage of reads that cross any part of the target region vs total reads. A read that partially maps to a target region is counted as on target.	%
SCALED_MEDIAN_ GENE_COVERAGE	Median of median base coverage of genes scaled by length. An indication of median coverage depth of genes in the panel.	Count
TOTAL_PF_READS	Total number of reads passing filter	Count

Appendix C TruSight Oncology Comprehensive (EU) Report Reference



A Refer to *Appendix A QC Metrics Flowchart* on page 39 for details.

B 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 14. If there are no CDx results, the report states that no Companion Diagnostic biomarkers for the stated sample tumor type were detected.

C 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.

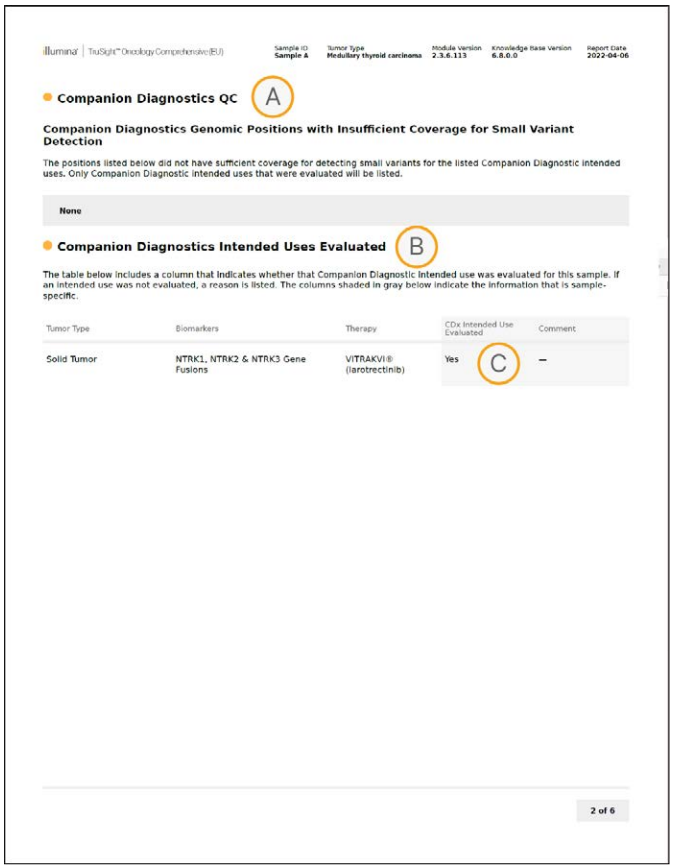
D The Other Alterations and Biomarkers Identified section contains tumor profiling information. Associations can be due to therapeutic, diagnostic, or prognostic evidence. If applicable, this section also lists resistance mutations with a corresponding note.

E According to the KB, there is evidence of clinical significance for this biomarker in this tumor type based on information from therapy, clinical guidelines, or both. For more information, refer to *Genomic Findings with Evidence of Clinical Significance* on page 15 and the Genomic Findings with Evidence of Clinical Significance table on page 21.

F According to the KB, there is limited or no clinical evidence for a genomic finding within the tumor type. There might be preclinical data or data in other tumor types where the biomarker is predictive of response to an approved or investigational therapy. For more information, refer to and the *Genomic Findings with Potential Clinical Significance* on page 16 and the Genomic Findings with Potential Clinical Significance table on page 22

G TMB and MSI are listed in Genomic Findings with Potential Clinical Significance. Refer to *Tumor Mutational Burden* on page 11 and *Microsatellite Instability Status* on page 11.

H If there are two variants listed in a single row (not pictured), there is clinical meaning for these variants when they are detected together. Resistance mutations or other sources can be the cause. Refer to examples in *Tumor Profiling of Variants* on page 15.



A The Companion Diagnostic QC section provides position-level QC information about CDx biomarkers. If no positions are listed, it means that there was sufficient coverage throughout the targeted variants and region. For more information, refer to the Companion Diagnostics QC table on page 22.

B 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 (EU) Package Insert (document # 200007789) for more information about the TSO Comprehensive assay intended use. Tumor type, Biomarker, and Therapy are from the Intended Use statement.

C Evaluation occurs if the tumor type is appropriate for a CDx and the sample passed required QC categories. For more on information on criteria required for samples to be evaluated for a CDx, refer the Companion Diagnostics Intended Uses Evaluated table on on page 23.

- Yes—The sample was evaluated for this intended use. Specific results would be identified in the Companion Diagnostics Results section of the report.
- No—The sample was not evaluated for this intended use and a comment explains why.

Appendix D MNVs, Indels, and Deletions in EGFR and RET Detectable by Phased Variant Caller

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr7	55242462	CAAGGAATTAAGAGAA	C	EGFR	NP_005219.2:p.(Lys745_Glu749del)
chr7	55242463	AAGGAATTAAGAGAAG	A	EGFR	NP_005219.2:p.(Lys745_Ala750delinsThr)
chr7	55242464	AGGAATTAAGAGA	A	EGFR	NP_005219.2:p.(Glu746_Glu749del)
chr7	55242464	AGGAATTAAGAGAAGC	A	EGFR	NP_005219.2:p.(Glu746_Ala750del)
chr7	55242465	GGAATTAAGA	G	EGFR	NP_005219.2:p.(Leu747_Glu749del)
chr7	55242465	GGAATTAAGAGAAG	AATTC	EGFR	NP_005219.2:p.(Glu746_Ala750delinsIlePro)
chr7	55242465	GGAATTAAGAGAAGCAA	AATTC	EGFR	NP_005219.2:p.(Glu746_Thr751delinsIlePro)
chr7	55242465	GGAATTAAGAGAAGCAAC	AAT	EGFR	NP_005219.2:p.(Glu746_Thr751delinsIle)
chr7	55242465	GGAATTAAGAGAAGCAACA	G	EGFR	NP_005219.2:p.(Glu746_Thr751del)
chr7	55242465	GGAATTAAGAGAAGCAACATC	AAT	EGFR	NP_005219.2:p.(Glu746_Ser752delinsIle)
chr7	55242465	GGAATTAAGAGAAGCA	G	EGFR	NP_005219.2:p.(Glu746_Ala750del)
chr7	55242466	GAATTAAGAGAAGCAACAT	G	EGFR	NP_005219.2:p.(Glu746_Ser752delinsAla)
chr7	55242466	GAATTAAGAGAAGCAA	G	EGFR	NP_005219.2:p.(Glu746_Thr751delinsAla)
chr7	55242467	AATTAAGAGAAGCAAC	A	EGFR	NP_005219.2:p.(Leu747_Thr751del)
chr7	55242467	AATTAAGAGAAGCAACATC	A	EGFR	NP_005219.2:p.(Glu746_Ser752delinsAsp)
chr7	55242467	AATTAAGAGAAGCAACATC	T	EGFR	NP_005219.2:p.(Glu746_Ser752delinsVal)
chr7	55242467	AATTAAGAGAAGCAACATCTC	TCT	EGFR	NP_005219.2:p.(Glu746_Pro753delinsValSer)
chr7	55242467	AATTAAGAGAAGCAACA	TTGCT	EGFR	NP_005219.2:p.(Glu746_Thr751delinsValAla)
chr7	55242467	AATTAAGAGAAGCAAC	T	EGFR	NP_005219.2:p.(Glu746_Thr751delinsVal)
chr7	55242468	ATTAAGAGAAGCAACATCT	A	EGFR	NP_005219.2:p.(Leu747_Ser752del)
chr7	55242468	ATTAAGAGAAGCAAC	GCA	EGFR	NP_005219.2:p.(Leu747_Thr751delinsGln)
chr7	55242468	ATTAAGAGAAG	GC	EGFR	NP_005219.2:p.(Leu747_Ala750delinsPro)
chr7	55242469	TTAAGAGAAG	C	EGFR	NP_005219.2:p.(Leu747_Ala750delinsPro)
chr7	55242469	TTAAGAGAAGCAA	C	EGFR	NP_005219.2:p.(Leu747_Thr751delinsPro)
chr7	55242469	TTAAGAGAAGCAACATCT	CAA	EGFR	NP_005219.2:p.(Leu747_Ser752delinsGln)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr7	55242469	TTAAGAGAAGCAACATCTCC	CA	EGFR	NP_005219.2:p.(Leu747_Pro753delinsGln)
chr7	55242469	TTAAGAGAAGCAACATCTC	T	EGFR	NP_005219.2:p.(Leu747_Pro753delinsSer)
chr7	55242469	TTAAGAGAAGCAA	T	EGFR	NP_005219.2:p.(Leu747_Thr751delinsSer)
chr7	55242482	CATCTCCGAAAGCCAACAAGGAAAT	C	EGFR	NP_005219.2:p.(Ser752_Ile759del)
chr7	55249011	AC	CCAGCGTGGAT	EGFR	NP_005219.2:p.(Ala767_Val769dup)
chr10	43604549	CTCAGACTTCCAGGGCCCAGGA	G	RET	NP_066124.1:p.(Asp378_Gly385delinsGlu)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CACAC	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CACAT	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CCCAC	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CCCAT	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CGCAC	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CGCAT	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CTCAC	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609928	ATCCACTGTGCGACGAGCTG	CTCAT	RET	NP_066124.1:p.(Asp627_Leu633delinsAlaHis)
chr10	43609933	CTGTGCGACGAGCTGTGCCGCACGGTGATC	TGCGAT	RET	NP_066124.1:p.(Leu629_Ile638delinsCysAsp)
chr10	43609933	CTGTGCGACGAGCTGTGCCGCACGGTGATC	TGTGAT	RET	NP_066124.1:p.(Leu629_Ile638delinsCysAsp)
chr10	43609933	CTGTGCGACGAGCTGTGCCGCACGGTGAT	TGCGA	RET	NP_066124.1:p.(Leu629_Ile638delinsCysAsp)
chr10	43609933	CTGTGCGACGAGCTGTGCCGCACGGTGAT	TGTGA	RET	NP_066124.1:p.(Leu629_Ile638delinsCysAsp)
chr10	43609936	TGC	GCT	RET	NP_066124.1:p.(Cys630Ala)
chr10	43609940	ACGAGCTG	TA	RET	NP_066124.1:p.(Asp631_Leu633delinsVal)
chr10	43609940	ACGAGCTG	TC	RET	NP_066124.1:p.(Asp631_Leu633delinsVal)
chr10	43609940	ACGAGCTGTGCCGCACGGTGAT	C	RET	NP_066124.1:p.(Asp631_Ile638delinsAla)
chr10	43609940	ACGAGCTGTGCCGCACGGTGATC	CA	RET	NP_066124.1:p.(Asp631_Ile638delinsAla)
chr10	43609940	ACGAGCTGTGCCGCACGGTGATC	CG	RET	NP_066124.1:p.(Asp631_Ile638delinsAla)
chr10	43609940	ACGAGCTGTGCCGCACGGTGATC	CT	RET	NP_066124.1:p.(Asp631_Ile638delinsAla)
chr10	43609940	ACGAGCTG	TT	RET	NP_066124.1:p.(Asp631_Leu633delinsVal)
chr10	43609941	CGAGCTG	A	RET	NP_066124.1:p.(Asp631_Leu633delinsGlu)
chr10	43609942	GAGCTGTGCCGCA	AGCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609942	GAGCTGTGCCGCA	AGTT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGCAGC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGCAGT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGCTCA	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGCTCC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGCTCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGTAGC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGTAGT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGTTCA	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGTTCC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	AGTTCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CACAGG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CACCGC	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CACCGG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CACCGT	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CATAGG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CATCGC	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CATCGG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGCA	CATCGT	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGC	CACAG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGC	CACCG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGC	CATAG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACGGTGATCGC	CATCG	RET	NP_066124.1:p.(Glu632_Ala639delinsHisArg)
chr10	43609942	GAGCTGTGCCGCACG	TCAAGC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCAAGT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCATCA	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCATCC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCATCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609942	GAGCTGTGCCGCACG	TCCAGC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCCAGT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCCTCA	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCCTCC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCCTCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCGAGC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCGAGT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCGTCA	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCGTCC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCGTCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCTAGC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCTAGT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCTTCA	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCTTCC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCACG	TCTTCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCA	TCAT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCA	TCCT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCA	TCGT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609942	GAGCTGTGCCGCA	TCTT	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)
chr10	43609943	AGCTG	TA	RET	NP_066124.1:p.(Glu632_Leu633delinsVal)
chr10	43609943	AGCTG	TC	RET	NP_066124.1:p.(Glu632_Leu633delinsVal)
chr10	43609943	AGCTGTGCCGCACGGT	CAGC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGT	CCGC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGT	CGGC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGT	CTGC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TAAGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TAAGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TAAGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TAAGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TAAGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGCCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGCCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGCCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGGCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGTCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGTCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TACGTCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCAGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCAGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCAGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCAGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCAGGCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCAGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGCCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGCCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGCCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGGCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGTCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGTCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TCCGTCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGAGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGAGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGAGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGAGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGAGGCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGAGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGCCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGCCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGCCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGTCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGTCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TGCGTCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTAGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTAGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTAGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTAGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTAGGCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTAGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGACCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGACCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGCCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGCCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGCCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGGCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGGCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGGCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGTCCA	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGTCCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGTCCT	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TAAGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TAAGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TACGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TACGCC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TACGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TACGTC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TCAGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TCAGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TCCGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TCCGCC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TCCGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TCCGTC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TGAGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TGAGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TGCGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TGCGCC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TGCGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TGCGTC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TTAGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TTAGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TTCGAC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TTCGCC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TTCGGC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAG	TTCGTC	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)
chr10	43609943	AGCTGTGCCGCACGGTG	CAGCA	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CAGCC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CAGCT	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CCGCA	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CCGCC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CCGCT	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CGGCA	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CGGCC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CGGCT	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CTGCA	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CTGCC	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTGTGCCGCACGGTG	CTGCT	RET	NP_066124.1:p.(Glu632_Val637delinsAlaAla)
chr10	43609943	AGCTG	TT	RET	NP_066124.1:p.(Glu632_Leu633delinsVal)
chr10	43609944	GCTGT	CGTAC	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGT	CGTCC	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGT	CGTGC	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGT	CGTTC	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTAAGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609944	GCTGTGC	CGTAAGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTACGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTACGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTACGT	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTCAGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTCAGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTCCGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTCCGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTCCGT	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTGAGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTGAGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTGCGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTGCGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTGCGT	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTTAGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTTAGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609944	GCTGTGC	CGTTCGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTTCGG	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	CGTTCGT	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTAAGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTAAGG	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTACGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTACGG	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTACGT	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTCAGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTCCGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTCCGG	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTCCGT	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTGAGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTGAGG	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTGCGA	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTGCGG	RET	NP_066124.1:p.(Glu632_ Cys634delinsAspValArg)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609944	GCTGTGC	TGTGCGT	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTTAGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTTAGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTTCGA	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTTCGG	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGTGC	TGTTCGT	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGT	TGTAC	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGT	TGTCC	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGT	TGTGC	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609944	GCTGT	TGTTC	RET	NP_066124.1:p.(Glu632_Cys634delinsAspValArg)
chr10	43609945	CTGTGC	GTATGG	RET	NP_066124.1:p.(Leu633_Cys634delinsValTrp)
chr10	43609945	CTGTGC	GTCTGG	RET	NP_066124.1:p.(Leu633_Cys634delinsValTrp)
chr10	43609945	CTGTGC	GTGTGG	RET	NP_066124.1:p.(Leu633_Cys634delinsValTrp)
chr10	43609945	CTGTGC	GTTTGG	RET	NP_066124.1:p.(Leu633_Cys634delinsValTrp)
chr10	43609948	TGC	CCA	RET	NP_066124.1:p.(Cys634Pro)
chr10	43609948	TGC	CCG	RET	NP_066124.1:p.(Cys634Pro)
chr10	43609950	CCGC	GGGA	RET	NP_066124.1:p.(Cys634_Arg635delinsTrpGly)
chr10	43609950	CCGC	GGGG	RET	NP_066124.1:p.(Cys634_Arg635delinsTrpGly)
chr10	43609950	CCGC	GGGT	RET	NP_066124.1:p.(Cys634_Arg635delinsTrpGly)
chr10	43609950	CCGC	TCCAAAAAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAAAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609950	CCGC	TCCAAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAGAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAGAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCAAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAAAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAAAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAGAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAGAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCCAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAAAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAAAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAGAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAGAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCGAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609950	CCGC	TCCTAAAAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAAAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAGAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAGAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	CCGC	TCCTAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCAAAA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCAAAG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCCAAA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCCAAG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCGAAA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCGAAG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCTAAA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609950	C	TCCTAAG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAAAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAGAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CAAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAAAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609952	GC	CCAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAGAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAGAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CCAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAAAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAAAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAGAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAGAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CGAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAAAGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAACGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAACGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAACGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAGAGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAGCGA	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAGCGG	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43609952	GC	CTAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
chr10	43613904	TTG	ACT	RET	NP_066124.1:p.(Leu790Thr)
chr10	43615630	TTCC	ACCA	RET	NP_066124.1:p.(Asp903_Ser904delinsGluPro)
chr10	43615630	TTCC	ACCG	RET	NP_066124.1:p.(Asp903_Ser904delinsGluPro)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43615630	TTCC	ACCT	RET	NP_066124.1:p.(Asp903_Ser904delinsGluPro)
chr10	43615630	TTCC	GCCA	RET	NP_066124.1:p.(Asp903_Ser904delinsGluPro)
chr10	43615630	TTCC	GCCG	RET	NP_066124.1:p.(Asp903_Ser904delinsGluPro)
chr10	43615630	TTCC	GCCT	RET	NP_066124.1:p.(Asp903_Ser904delinsGluPro)

Revision History

Document	Date	Description of Change
Document # 200008661 v03	July 2022	Added TSO Comp v2.3.5 security certification information. Updated Module Settings screen name to Modules & Manifests.
Document # 200008661 v02	April 2022	Added companion diagnostic content. Added NTRK clinical study content.
Document # 200008661 v01	February 2022	Added DNA and RNA Expanded Metrics sections.
Document # 200008661 v00	November 2021	Initial release.

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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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