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Local Run Manager TruSight Oncology Comprehensive (US) Analysis Module

Workflow Guide

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Overview

The Illumina® Local Run Manager TruSight™ Oncology Comprehensive (US) Analysis Module (TSO Comprehensive (US) analysis module) analyzes sequencing reads of DNA and RNA libraries prepared using the TruSight Oncology Comprehensive (TSO Comprehensive) assay. Refer to the *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)* for the TSO Comprehensive assay intended use.

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

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

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

The TSO Comprehensive (US) analysis module comprises the TSO Comprehensive Software Suite, a Knowledge Base (KB), and a TSO Comprehensive Claims Package. The KB and the TSO Comprehensive Claims Package are installed into the TSO Comprehensive (US) analysis module. For part numbers and version numbers, refer to *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)*.

About This Guide

This guide provides instructions for setting up run parameters for sequencing and analysis using the TSO Comprehensive (US) 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 TSO Comprehensive (US) analysis module dashboard and system settings, refer to the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Enter Run Information

Use the Local Run Manager TSO Comprehensive (US) Analysis Module software to set up TSO Comprehensive runs.

Before beginning the run, make sure that a compatible KB is installed. If a compatible KB is not installed, refer to *Appendix E Install a Knowledge Base* on page 97.

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

TSO Comprehensive Analysis Module Information

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

1. Open TSO Comprehensive (US) analysis module on your instrument.
2. Use the Tools menu to navigate to the Modules & Manifests screen.
3. Select **TSO Comp (US)**.

The Modules & Manifests screen displays the following installation information:

- **Device Identifier**—A unique device identifier for the installed TSO Comprehensive (US) analysis module and associated Claims Package. The installed KB version does not impact this identifier.
- **Product Identifier**—The version of the installed TSO Comprehensive (US) analysis module.
- **Modified On**—The date and time that the TSO Comprehensive (US) 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 (US) 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 the TSO Comprehensive (US) analysis module evaluates.
- **TSO Comprehensive (US) Security Certificate**—HTTPS certificate specific to this instrument. Required for remote access using a web browser of this instrument from another machine in the same network. Refer to *Appendix F Cybersecurity* on page 99 for installation instructions.
- **Knowledge Base Version**—Refer to *Appendix E Install a Knowledge Base* on page 97 for instructions on installing or updating the KB. This section includes KB installation information for the following fields:

Field	Description
Name	KB name
Version	KB version
RefSeq Version	RefSeq version included in the KB. For CDx annotation, the RefSeq transcripts originate from the Ensembl Variant Effect Predictor (VEP)[1], and the VEP version is displayed. For tumor profiling annotation, the RefSeq version shown indicates which NCBI Homo sapiens Annotation Release[2] it originates from.
Published	KB publication date
Installed	KB installation date
State	KB installation State. Displays as Ready when installation is complete.

[1] McLaren W, Gil L, Hunt SE, et al. The ensembl variant effect predictor. Genom Biol. 2016 Jun 6, 17(1):122.g.
[2] NCBI Homo sapiens Updated Annotation Release 105.20201022. https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Homo_sapiens/105.20201022.

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 (US)**.
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.
   - An alphanumeric character must precede and follow dashes or underscores.
   - 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.
   - An alphanumeric character must precede and follow spaces.

Specify Samples for the Run

Specify samples for the run using the following options:

- **Enter samples manually**—Use the blank table at the bottom of the Create Run screen.
- **Import sample sheet**—Navigate to an external file in a comma-separated values (*.csv) format.

Enter Samples Manually

1. Enter a unique sample ID in the Sample ID field with the following criteria. **Add all controls before intended use samples.** Refer to *Controls* on page 5 for more information.
   - 1–25 characters.
   - Only alphanumeric characters, underscores, or dashes.
   - An alphanumeric character must precede and follow dashes or underscores.
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.
   - An alphanumeric character must precede and follow dashes, spaces, or underscores.
3. Select an index for the DNA library and/or RNA library prepared from the sample. Refer to the *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)* for number of libraries and index ID selection.
   - Make sure that RNA and DNA samples are in separate columns.
   - The DNA i7+i5 Sequence field autopopulates after selecting a DNA Index ID. The RNA i7+i5 Sequence field autopopulates after selecting an RNA Index ID.
   - 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 (US) Package Insert (document # 200061832)*.
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 5.
5. Use the Tumor Type field to assign one of the following control types for each control. Refer to *Controls* on page 5.
   - DNA External Control (DNA Positive Control)
   - DNA No-Template Control
   - RNA External Control (RNA Positive Control)
   - RNA No-Template Control
6. Assign Sex. For Controls, Sex is Unknown.
7. **[Optional]** Select **Export to CSV** to export sample information to a 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 that 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 7). The Tumor Type field is also used to designate samples as controls (refer to *Controls* on page 5).
   - Use the file of sample information that was exported from the TSO Comprehensive (US) 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**.

Controls

TSO Comprehensive requires the use of TruSight Oncology DNA and RNA Controls. Designating a sample as a control automatically sets the Sex of the sample to Unknown. To designate a control sample, select one of four control types from the Tumor Type field:

- DNA External Control (positive DNA control)
- RNA External Control (positive RNA control)
- DNA No-Template Control
- RNA No-Template Control

Refer to *Select a Tumor Type* on page 5 for more information on setting tumor types for all types of samples during run setup.

Specify one of each control type within a run. Select a DNA library for a DNA External Control or a DNA No-Template Control. Select an RNA library for an RNA External Control or an RNA No-Template Control. DNA or RNA No-Template controls are not counted against the maximum number of libraries in a run.

Refer to the *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)* for more information on using control samples.

Select a Tumor Type

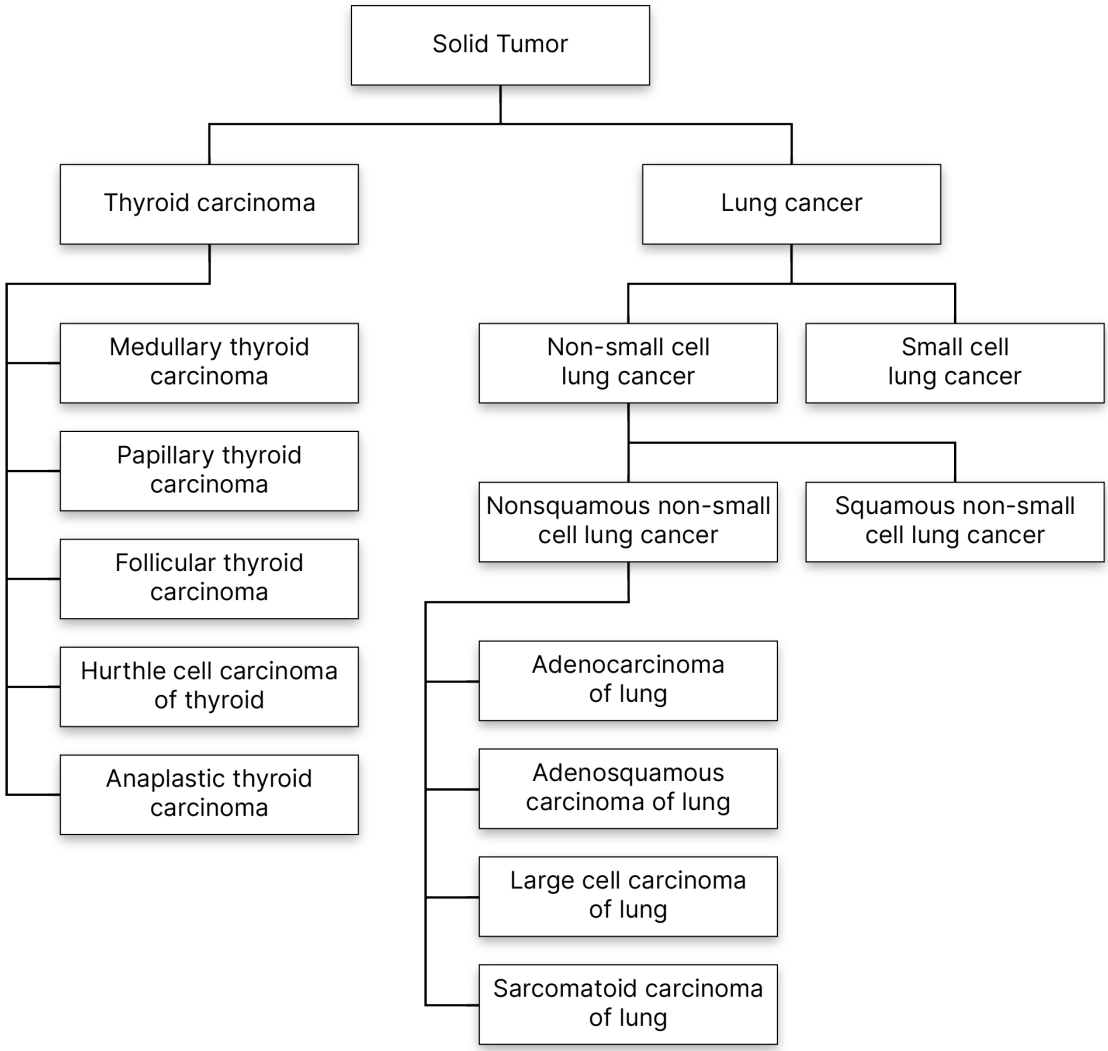
A tumor type must be specified for each sample. Except for control types, the available tumor types are derived from the installed KB and might change with updated versions of the KB.

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 because 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 Example of a Disease Ontology Subset



The selected tumor type for a patient sample impacts:

- Which companion diagnostic intended uses are evaluated for the sample. Only patient samples with a tumor type that is an exact match or a descendant of the cancer type for a companion diagnostic intended use are evaluated for that claim.
- Which tumor profiling variants are included in the TSO Comprehensive report. Refer to *Tumor Profiling of Variants* on page 16.

Select a tumor type using 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 5).

1. Double-click the Tumor Type cell to view the available tumor types. Available tumor types are displayed in an alphabetized hierarchical list. The Tumor Type field is also used to designate a control type for control samples (refer to *Controls* on page 5).
2. Use the list or search bar at the top of the Tumor Type window to select the desired tumor type.

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 (US) 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 begin after 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 collecting the sequencing data, the TSO Comprehensive (US) analysis module processes it to:

- Perform quality control.
- Detect variants.
- Determine Tumor Mutational Burden (TMB).
- Determine companion diagnostic results.
- Assess the clinical significance and potential clinical significance of detected variants.
- 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 is reported as PASS and analysis continues. If a value for any one of the metrics fails to meet the specification, then Run QC is reported as FAIL and analysis does not proceed. For more information, refer to *Quality Control Metrics* on page 60.

FASTQ Generation

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

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

DNA Alignment and Error Correction

DNA alignment and error correction involve aligning sequencing reads derived from DNA sample libraries to a reference genome and correcting errors in the sequencing reads before 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 BAM files are further processed to remove errors (including errors introduced during PCR amplification or sequencing). Reads derived from the same unique DNA molecule are collapsed into a single representative sequence, using their unique molecular identifier (UMI) incorporated into the library fragments during library preparation.

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 (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 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), insertions, 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 67) 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. gVCF files 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 artifacts from sample processing (such as deamination or oxidation). 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 samples matching the intended use population of varying qualities through the TSO Comprehensive assay. To address sample-specific artifacts, the reads supporting the variant call are stratified by error rate. Reads originating from duplex/stitched reads have the lowest error rate and reads originating from simplex (nonduplex/unstitched) reads have 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. True somatic events, because of their relative rarity, do 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 that have low adjusted quality scores are tagged with a LowSupport filter flag and are considered as reference calls. If 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 identifies 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 (in phase with each other). Overlapping reads are clustered in the neighborhood into a minimal set of clusters that 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 67. MNVs, indels, and deletions from the phased variant caller take precedence over those that may 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 and various population databases (COSMIC, ClinVar, dbSNP, 1000 Genomes, and gnomAD). Annotation of small variants is performed multiple times independently as described in the following sections.

Static Annotation Databases for TMB Calculation

Nirvana annotates 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 is used as input (refer to *Small Variant Calling* on page 9). Variants detected by the phased variant caller are not used for TMB calculation.

Static Annotation Databases for Companion Diagnostic Calling

Nirvana annotates filtered small variant calls with static (not updatable) annotation databases for use by downstream Companion Diagnostic calling (refer to *Companion Diagnostic Calling* on page 15). The gVCF from the Small Variant Phasing step is used as input (refer to *Small Variant Calling* on page 9).

Updatable RefSeq Database for Tumor Profiling

Nirvana annotates 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 16). The updatable RefSeq database is included as part of the KB and may be updated periodically to be compatible with other KB content.

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. TSO Comprehensive does not differentiate between variants of somatic or germline origin for purposes of small variant calling. Variants are flagged as likely germline for calculating the TMB score, applying 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 proximity filter labels variants as germline if they are surrounded 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.

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. If both the contamination score and rearrangement p-value are above

predefined quality thresholds, a sample is considered to be contaminated. If contamination is detected, then DNA Library QC is reported as FAIL and no results are available for small variants and TMB. Also, a companion diagnostic or tumor profiling result is not available if it relies on DNA library QC passing.

QC metrics are used to assess the validity of small variant calling and TMB 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. The associated QC category in the report header displays as FAIL. Also, 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 48.

Low Depth Reporting for DNA Sample Libraries

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

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

Refer to *Low Depth Report* on page 51 for details on the content.

RNA Alignment

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

1. First, RNA sequences in FASTQ files are downsampled to approximately 30 million reads per RNA sample library. Downsampling 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.
2. Preprocessed reads are then aligned to the hg19 reference genome and candidate splice junctions are identified. This step generates BAM files and BAM index files for aligned reads, and a tab-delimited text file for candidate splice junctions.
3. 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 (reads aligning to different chromosomes or in unexpected orientations) in the BAM files (generated during RNA Alignment) for the fusion genes targeted by TSO Comprehensive. 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 various filters before 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. This filter primarily 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 are 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 14).

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 14).

RNA Fusion Merging

Fusions identified during RNA Fusion Calling are merged with fusions from proximal genes identified during RNA Splice Variant Calling. The merged fusions are then annotated with gene symbols or names corresponding 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 annotates detected RNA splice variant calls with a static (not updatable) RefSeq database for use by downstream Companion Diagnostic calling (refer to *Companion Diagnostic Calling* on page 15). Splice variants are annotated with transcript-level changes (affected exons in the gene 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 annotates 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 16). Splice variants are annotated with transcript-level changes (affected exons in the gene 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 is reported as FAIL and no results are available for fusions or splice variants. Also, a companion diagnostic or tumor profiling result is not 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 48.

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 (for example, 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. The first letter indicates the wild type allele and the second letter indicates 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 included in the run. The TSO Comprehensive (US) analysis module automatically invalidates patient samples based on control sample results.

Refer to *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)* for guidance on run validity and patient sample validity based on results for controls.

The control output report is available in the `ControlOutput.csv` file. Refer to *Control Output Report* on page 44.

Companion Diagnostic Calling

For each installed companion diagnostic (CDx) intended use, the TSO Comprehensive (US) analysis module determines the applicability of the CDx intended use for each patient sample based on the tumor type of the patient sample. If the tumor type of the patient is an exact match or a descendant of the tumor type for a CDx intended use, it is considered applicable to that CDx intended use. Refer to *Select a Tumor Type* on page 5 for more information on the disease ontology. If the tumor type of the patient is not applicable to a CDx intended use, then the CDx intended use is not evaluated for that sample.

If a required sequencing library (DNA or RNA) for a CDx intended use is not sequenced or, fails QC, or required controls in the run fail, then the patient sample is not evaluated for that CDx intended use. If a variant type (such as small variants) or biomarker required for a CDx intended use fails QC, then the patient sample is not evaluated for that CDx intended use.

When it is determined that a CDx intended use is applicable for a patient sample, the required libraries are sequenced, required QC measures pass, and required controls pass, the companion diagnostic intended use is evaluated for the patient sample. Detected variants and/or biomarkers in the patient sample are evaluated to determine the result for the CDx intended use. The evaluation is done through an algorithm specific to the CDx intended use, which assesses 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 (refer to *TruSight Oncology Comprehensive Report* on page 20). 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 cancer mutations that have evidence of clinical significance or have potential clinical significance. This process is called Tumor Profiling of Variants. A cancer mutation 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 findings, then that variant may be listed more than one time on a report. A genomic finding with a single variant will only be listed at the highest level where it meets criteria for reporting. Refer to *Positive CDx Results* on page 18 for additional details. The following examples of genomic findings and clinical meanings include multiple variants, for illustrative purposes only. For example, the genomic finding levels may vary depending on tumor type, as discussed later in this section.

- A genomic finding of a single variant NTRK1 p.(G595R) 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 two genomic findings, RET p.(D898_E901del) and RET p.(D903_S904delinsEP). The patient exhibited tumor response to treatment with a RET inhibitor (PMID 32846061).
- A BRAF p.(V600E) mutation co-occurring with TERT promoter mutation is associated with an unfavorable prognosis in papillary thyroid carcinoma per major US guidelines.

Cancer Mutations with Evidence of Clinical Significance

Cancer Mutations with evidence of clinical significance are reported in the Cancer Mutations with Evidence of Clinical Significance section of the TSO Comprehensive report (refer to *TruSight Oncology Comprehensive Report* on page 20). Cancer mutations are reported in this section if they meet the following criteria:

- The genomic finding is associated with benefit or lack of benefit to a therapy, as evidenced by an FDA-approved drug label. The tumor type of the sample 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 5 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 published ASCO guidelines or other major US clinical practice guidelines. 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 5 for more information on the disease ontology.

Cancer Mutations with Potential Clinical Significance

Cancer mutations with potential clinical significance are reported in the Cancer Mutations with Potential Clinical Significance section of the TSO Comprehensive report (refer to *TruSight Oncology Comprehensive Report* on page 20). Cancer mutations are reported in this section if they meet the following criteria:

- The genomic finding meets criteria for cancer mutations with evidence of clinical significance (for example, FDA-approved drug label, ASCO guidelines, or other major US guidelines), but only when the tumor type of the sample is not a match to the KB association's tumor type. The tumor type of the sample 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 tumor type of the sample 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. The tumor type of the sample must be equal to or a descendant of the clinical trial's tumor type.

TMB score is always reported in the Cancer Mutations with Potential Clinical Significance section of the TSO Comprehensive report, regardless of the tumor type of the sample.

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 the Cancer Mutations with Evidence of Clinical Significance or Cancer Mutations with Potential Clinical Significance sections of the TSO Comprehensive report through a KB content update. Likewise, variants may move from the Cancer Mutations with Evidence of Clinical Significance or Cancer Mutations with Potential Clinical Significance sections of the TSO Comprehensive report or vice versa when KB content is updated. 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 Cancer Mutations with Evidence of Clinical Significance and Cancer Mutations with Potential Clinical Significance. However, cancer mutations involving multiple variants might still be reported in Cancer Mutations with Evidence of Clinical Significance and Cancer Mutations with Potential Clinical Significance, even if one of the variants is reported in Companion Diagnostic Results.

COSMIC Annotations

Variants reported in the Cancer Mutations with Evidence of Clinical Significance or Cancer Mutations with Potential Clinical Significance sections of the TSO Comprehensive report 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 TSO Comprehensive (US) 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 is formatted as `Analysis_#` where # defaults to 1 and increments 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 (for example, `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 the Cancer Mutations with Evidence of Clinical Significance or Cancer Mutations with Potential Clinical Significance sections of the TSO Comprehensive report are not included in the reports.

Refer to the *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)* 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.

Table 1 Sample, Run, and Analysis Information

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.
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 58.

Table 2 Quality Control

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 Small Variant, TMB QC, DNA External Control & NTC, RNA External Control & NTC, and RNA Library QC) and there are no variants or biomarkers listed in the report. Refer to the TruSight Oncology Comprehensive (US) Package Insert (document # 200061832) for guidance on run validity and patient sample validity based on results for controls.
RNA External Control & NTC	qualityControl/status/ (array item having label = "RNA External Control & NTC")	RNA External Control & NTC Control Results (PASS, FAIL, or N/A) applies to the RNA library that was sequenced. • **PASS**—both the RNA External Control and RNA No-Template Control have a result of PASS. • **FAIL**—the RNA External Control and/or RNA No-Template Control has a result of FAIL. • **N/A**—the RNA library for the sample was not sequenced or reanalyzed during requeue, the RNA External Control or NTC for the run was not sequenced or reanalyzed during requeue, 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.

Field in PDF report	Field in JSON report	Description
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 External Control & NTC	qualityControl/status/ (array item having label = "DNA External Control & NTC")	DNA External Control & NTC Control Results (PASS, FAIL, or N/A) applies to the DNA library that was sequenced. • **PASS**—both the DNA External Control and DNA No-Template Control have a result of PASS. • **FAIL**—the DNA External Control and/or DNA No-Template Control has a result of FAIL. • **N/A**—the DNA library for the sample was not sequenced or reanalyzed during requeue, the DNA External Control or NTC for the run was not sequenced or reanalyzed during requeue, or Run QC had a value of FAIL. If the value is FAIL or N/A, there are no DNA variant types (small variants) or DNA biomarkers (TMB) 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) or DNA biomarkers (TMB) are reported.

Field in PDF report	Field in JSON report	Description
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.

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

Table 3 Analysis Module and KB Configuration

Field in PDF report	Field in JSON report	Description
Knowledge Base Version	softwareConfiguration / knowledgeBaseVersion	Version of the Knowledge Base installed with the TSO Comprehensive (US) 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 (US) analysis module used to generate the report.
Claims Package Version	softwareConfiguration / claimsPackageVersion	Version of the Claims Package installed with the TSO Comprehensive (US) 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 evaluated, are listed in the JSON report only. Refer to *Companion Diagnostics Intended Uses Evaluated* on page 30.

Table 4 Companion Diagnostic Results

Field in PDF report	Field in JSON report	Description
[Message box]	reportFindings / companionDiagnosticResults / results / noEntryText	**No Companion Diagnostic biomarkers for the stated sample tumor type were detected. See Companion Diagnostics Intended Uses Evaluated Table.** 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	**One or more Companion Diagnostic claims not evaluated. See Companion Diagnostics Intended Uses Evaluated Table.** This message is included when at least one CDx intended use applicable to the sample 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 30 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.

Field in PDF report	Field in JSON report	Description
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 *Small Variant Details in Report* on page 34, *Fusion Details in Report* on page 39, and *Splice Variant Details in Report* on page 40 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 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 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 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 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**—The following two sections contain tumor profiling information for detected variants categorized into Cancer Mutations with Evidence of Clinical Significance and detected variants categorized into Cancer Mutations with Potential Clinical Significance. TMB score is reported in Cancer Mutations with Potential Clinical Significance. Refer to *Tumor Profiling of Variants* on page 16 for details on how a level is determined for detected variants.
- **Cancer Mutations 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.

Table 5 Cancer Mutations with Evidence of Clinical Significance

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, for example, NRAS p.(Q61R). 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 exons (as applicable), for example, EGFR Exon (s) 2-7 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 *Small Variant Details in Report* on page 34, *Fusion Details in Report* on page 39, and *Splice Variant Details in Report* on page 40 for a list of variant detail fields.

- **Cancer Mutations with Potential Clinical Significance**—TMB score is 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.

Table 6 Cancer Mutations with Potential Clinical Significance

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 due to a QC failure, DNA External Control failure, DNA No-Template Control failure, or a DNA library for the sample was not sequenced. TMB is always included in the Cancer Mutations with Potential Clinical Significance section.
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, for example, NRAS p.(Q61R). 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 exons (as applicable), for example, EGFR Exon(s) 2-7 skipped.

Field in PDF report	Field in JSON report	Description
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 *Small Variant Details in Report* on page 34 for a list of variant detail fields.

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

Table 7 Companion Diagnostics QC

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.

- **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.

Table 8 Companion Diagnostics Intended Uses Evaluated

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.

Field in PDF report	Field in JSON report	Description
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.

Field in PDF report	Field in JSON report	Description
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 (Evaluated/Not Evaluated). 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 External Control & NTC • 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 External Control & NTC • RNA Library QC To be evaluated, the sample 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 5.

Field in PDF report	Field in JSON report	Description
Comment	reportFindings / companionDiagnosticResults / qualityControl / intendedUsesEvaluated / companionDiagnosticTable / entries / (array item for CDx intended use) / comment	If CDx Intended Use Evaluated field is Evaluated and there are no additional comments needed, this field displays a dash. If CDx Intended Use Evaluated field is Evaluated and there are additional comments to list, a comment such as the following 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 Not Evaluated, 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. Refer to *Appendix B QC Metrics* on page 60 for a list of QC that must pass for CDx intended use to be assessed.

- **Intended Use, Informatics Details, Limitations**—Contains general information about the test and a list of limitations.

Table 9 Intended Use, Informatics Details, Limitations

Field in PDF report	Field in JSON report	Description
Intended Use	about / description	Test description and intended use.
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.

Table 10 TruSight Oncology Comprehensive 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.

- **Details in Report**—Contains information about small variants, fusion variants, and splice variants.

Table 11 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.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
Protein Change	additionalInfo / (array item having label property = “Protein Change”) / value	Change to the protein reference sequence in HGVS nomenclature, as applicable.
Nucleotide Change	additionalInfo / (array item having label property = “Nucleotide Change”) / value	Change to the coding DNA reference sequence (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.
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.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
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 (for example, RefSeq).
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / bioType	An Ensembl biotype classification for the transcript.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / aminoAcids	The change in amino acids, as applicable (for example, G/D).
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / cdnaPos	cDNA position.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / codons	Codon sequence change (for example, gGt/gAt), as applicable.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / cdsPos	Coding sequence position, as applicable.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / exons	The exons 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.
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 (RefSeq transcript) in HGVS nomenclature, as applicable.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
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 12 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 12 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”)	Observed fusion breakpoint 1 in RNA. Chromosome:position format (hg19).
Breakpoint 2	additionalInfo / (array item having label property = “Breakpoint 2”)	Observed fusion breakpoint 2 in RNA. Chromosome:position format (hg19).
Fusion Supporting Reads	additionalInfo / (array item having label property = “Fusion Supporting Reads”)	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 genes 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.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
N/A	detailedGeneFusionData / partner2 / gene	Symbols or name (from GENCODE Release 19) of genes overlapping Breakpoint 2. 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.

Table 13 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 fusions include: **Splice Variant**
Affected Exons	additionalInfo / (array item having label property = "Affected Exons")	The exons affected by the splice variant, as applicable. For example, 4–6 would indicate that exons 4, 5, and 6 were affected.
Affected Introns	additionalInfo / (array item having label property = "Affected Introns")	The introns affected by the splice variant, as applicable. For example, 3 would indicate that intron 3 was affected.
Transcript	additionalInfo / (array item having label property = "Transcript")	Transcript ID (RefSeq).
Breakpoint Start	additionalInfo / (array item having label property = "Breakpoint Start")	Observed splice variant breakpoint start in RNA. Chromosome:position format (hg19).

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
Breakpoint End	additionalInfo / (array item having label property = “Breakpoint End”)	Observed splice variant breakpoint end in RNA. Chromosome:position format (hg19).
Splice Supporting Reads	additionalInfo / (array item having label property = “Splice Supporting Reads”)	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 (for example, RefSeq).
N/A	detailedSpliceVariantData / annotation / gene	Gene symbol.
N/A	detailedSpliceVariantData / annotation / affectedExons	The exons affected by the splice 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.

Field in PDF report	Field in JSON report (relative path in variant JSON object)	Description
N/A	detailedSpliceVariantData / annotation / affectedIntrons	The introns affected by the splice variant, and total intron count, as applicable. For example, 3/6 would indicate that intron 3 was affected and that this transcript contains 6 introns in total.
N/A	detailedSpliceVariantData / annotation / transcript	Transcript ID.

Sample Sheet

File name: `SampleSheet.csv`

For each analysis, the TSO Comprehensive (US) 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 causes adverse effects downstream, including incorrect results or analysis failure.

Table 14 Sample Sheet 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 controls.
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 controls that were included in the run.

- If controls in the run fail, the TruSight Oncology Report includes a message that one or more controls failed and to review the control output file for details.
- If any DNA External Control or DNA No-Template Control fails, no DNA variants (small variants) or biomarkers (TMB) are reported.
- If any RNA External Control or RNA No-Template Control fails, no RNA variants (fusions, splice variants) are reported.

Refer to *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)* for guidance on run validity and patient sample validity based on results for controls.

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

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

Table 15 Control Types

Field	Description
Control Type	The control type of the control. Possible values include: • DNA External Control • DNA No-Template Control • RNA External Control • RNA No-Template Control.
Sample_ID	Sample ID of the control. Value is (Not Run) if this control type was not included in the run.
AnalysisComplete	Indication of whether analysis completed for this control. Possible values include TRUE, FALSE, not applicable.
Overall Result	The QC result for the control. Possible values include PASS, FAIL, N/A.
Sensitivity Value	The calculated sensitivity value for the control. Represents the ratio of detected control variants to the total number of expected control variants in the control. Only applicable for the following control types: • DNA External Control • RNA External Control
Sensitivity Threshold	The minimum sensitivity value required for the control to have a QC result of PASS. Only applicable for the following control types: • DNA External Control • RNA External Control

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

Table 16 Analysis Details

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 (US) analysis module.
Pipeline Version	The version of the analysis pipeline/workflow.

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

Table 17 Sequencing Run Details

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 and whether any samples failed due to a software error.

Table 18 Analysis Status

Field	Description
Sample_ID	Sample ID of the control. Value is (Not Run) for control types not included in the run.
COMPLETED_ALL_STEPS	Indicates whether the control completed all steps of the analysis. Possible values include TRUE, FALSE, N/A. 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 the control DNA small variants in the DNA External Control (positive DNA control) that were detected or not detected (one row per control variant). N/A values are listed if the DNA External Control was not included in the sequencing run.

Table 19 Small Variants Truth Table Results

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

- **Splice Variants Truth Table Results**—Contains information on the control RNA splice variants in the RNA External Control that were detected or not detected (one row per control variant). N/A values are listed if the RNA External Control was not included in the sequencing run.

Table 20 Splice Variants Truth Table Results

Field	Description
Detected	Indicates whether the control RNA splice variant was detected in the control. Possible values include TRUE, FALSE, N/A.
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 the control RNA fusion variants in the RNA External Control that were detected or not detected (one row per control variant). N/A values are listed if the RNA External Control was not included in the sequencing run.

Table 21 Fusions Truth Table Results

Field	Description
Detected	Indicates whether the control RNA fusion variant was detected in the control. Possible values include TRUE, FALSE, N/A.
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. N/A values are listed if the DNA No-Template Control was not included in the sequencing run.

Table 22 DNA NTC Library QC Metrics

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. N/A values are listed if the RNA No-Template Control was not included in the sequencing run.

Table 23 RNA NTC Library QC Metrics

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.

Table 24 Metrics Output File Header

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 (US) 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 60 for metric descriptions and thresholds.

Table 25 Run QC Metrics

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 N/A.

- **Analysis Status**—Contains information on whether analysis was 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).

Table 26 Analysis Status

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.

Table 27 QC Metrics Sections for Patient Samples

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 60 for metric descriptions and thresholds.	DNA Library QC
DNA Library QC Metrics for Small Variant Calling and TMB	QC metrics used as a 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 60 for metric descriptions and thresholds.	DNA Small Variant & TMB QC

Section	Description	Corresponding QC Category in TSO Comprehensive Report
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 62 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 60 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 64 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. The file 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.

Table 28 Header Information

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.
Local Run Manager Module version	The version of the TSO Comprehensive (US) analysis module.

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

Table 29 Genomic Range List

Column	Description
Chrom	Chromosome.
Start	Start position (hg19).
End	End position (hg19).
Gene	One or more gene symbols 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}_TSOCompUSModule_KB{version}_Report.pdf`—TSO Comprehensive report (PDF format) per patient sample
    - `{SampleID}_TSOCompUSModule_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 pipeline/workflow. The TSO Comprehensive (US) 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 the **Edit** icon, and edit the output run folder file path.
   The file path leading up to the output run folder is editable. The output run folder name cannot be changed.
4. **[Optional]** Select the **Copy to Clipboard** icon to copy the output run folder file path.
5. Select the Sequencing Information tab to review run parameters and consumables information.
6. Select the Samples & Results tab to view the analysis report.
   - If analysis was requeued, select the appropriate analysis from the Select Analysis drop-down list.
7. **[Optional]** Select the **Copy to Clipboard** icon to copy the Analysis Folder file path.

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 48). Refer to *Quality Control Metrics* on page 60 for metric descriptions and thresholds.

Controls

Controls are designated in the Run Setup screen of the TSO Comprehensive (US) analysis module. Results for 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 type. Possible values are DNA External Control, DNA No-Template Control, RNA External Control, and RNA No-Template Control. The installed KB does not affect the available control types.

- **Analysis Complete?**—Possible values are TRUE and FALSE. Controls marked as TRUE in the Analysis Complete? column have completed control analysis. If a control is marked FALSE, a software error has occurred. Contact Illumina technical support for more information.
- **Outcome**—Possible values are PASS and FAIL. DNA and RNA controls are evaluated independently. Failure of DNA controls only invalidates DNA samples, while failure of RNA controls only invalidates RNA samples. Refer to the following table for outcome value interpretation:

Control 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 `MetricsOutput.tsv` file (refer to *Metrics Output* on page 48). 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.
- **RNA Library QC**—Possible values are PASS and FAIL. Indicates whether the sample passed or failed RNA library QC, which applies to the RNA Solid-FFPE 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.

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. Control samples cannot be selected for report regeneration. 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 status**—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 (US) analysis module version**—Report regeneration requires a successful analysis from a matching version of the TSO Comprehensive (US) analysis module.
- **TSO Comprehensive (US) KB version**—Report regeneration requires a successful analysis using a KB with a matching version of the RefSeq database.
- **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 TSO Comprehensive (US) analysis module admin users or a non-admin user with requeue analysis permissions assigned. For more information on TSO Comprehensive (US) analysis module 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 TSO Comprehensive (US) analysis module 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 are 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 controls, 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 TSO Comprehensive (US) analysis module. Reports produced using report regeneration are marked as Report Regeneration in the Analysis Type field at the top of the Samples and Runs screen.

Appendix A QC Metrics Flowchart

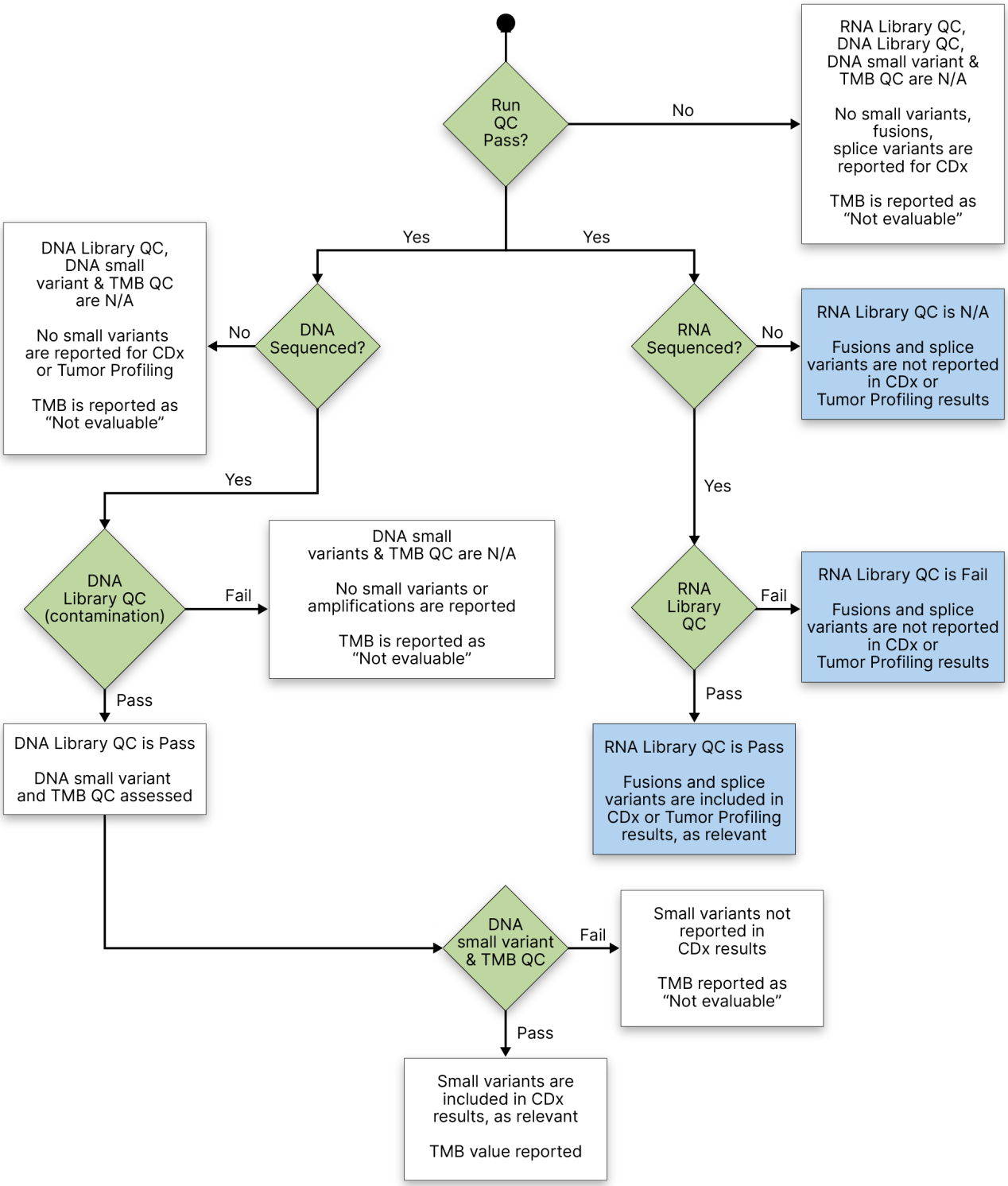
The following flowchart describes the QC metrics that are listed on the TSO Comprehensive report. If Run QC fails, then no other QC steps are assessed, and all are marked as N/A. If DNA or RNA is not sequenced or fail Library QC, then any corresponding variant types are not included in Companion Diagnostic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

- *Analysis Methods* on page 8
- *Quality Control* on page 22
- *Run QC Metrics* on page 48
- *Quality Control for DNA Sample Libraries* on page 11
- *Sample Level Metrics* on page 54
- *Appendix B QC Metrics* on page 60

The flowchart does not map the controls. The results from the controls do not impact the QC metrics on the TSO Comprehensive PDF or JSON report. Failure of controls invalidates sample results separate of QC results as described in *TruSight Oncology Comprehensive Report* on page 20. The use of controls is described in *Controls* on page 5. For additional controls information, refer to the *TruSight Oncology Comprehensive (US) Package Insert (document # 200061832)*.

The flowchart does not map the position-level QC results. These results are part of the Companion Diagnostic QC results, which are described in *Companion Diagnostics QC* on page 30. Position-level QC results for the Tumor Profiling section are provided in the Low Depth Report (refer to *Low Depth Reporting for DNA Sample Libraries* on page 12.

Figure 2 QC Metrics Flowchart



Appendix B QC Metrics

Quality Control Metrics

Table 30 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.	

Output Type	Metric	Specification	Description	Impact of Specification Failure*
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.	

Output Type	Metric	Specification	Description	Impact of Specification Failure*
RNA Libraries	MEDIAN_INSERT_SIZE (bp)	≥ 80	The median fragment length in the sample.	No fusion 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.

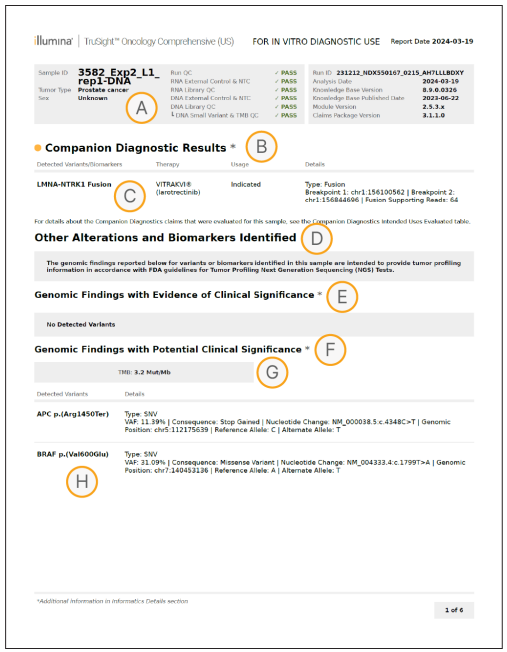
Metric	Description	Units
TOTAL_PF_READS	Total reads passing filter.	Count
MEAN_FAMILY_SIZE	The sum of the reads in each family divided by the number of families after correction, collapsing, and filtering on supporting reads.	Count
MEDIAN_TARGET_COVERAGE	The median coverage of bases.	Count
PCT_CHIMERIC_READS	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.	%
PCT_CONTAMINATION_EST	Percent of contamination of the sample.	%
PCT_PF_UQ_READS	Percent unique reads passing filter.	%
PCT_TARGET_0.4X_MEAN	Percent target bases with target coverage greater than 0.4 times the mean.	%
PCT_TARGET_100X	Percent target bases with greater than 100X coverage.	%
PCT_TARGET_250X	Percent target bases with greater than 250X coverage.	%

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 that 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 TSO Comprehensive (US) Report Reference



A. Refer to *Appendix A QC Metrics Flowchart* on page 58 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 15. 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 the 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.

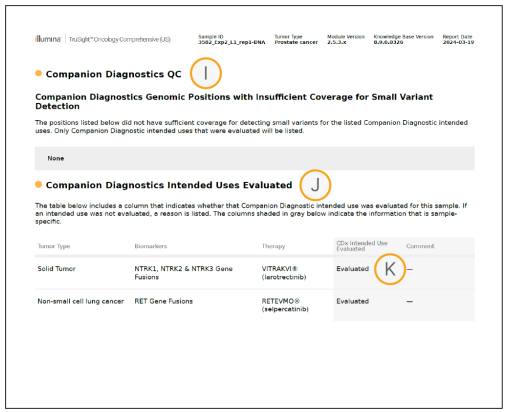
D. The Other Alterations and Biomarkers Identified section contains tumor profiling information. Associations can be due to therapeutic, diagnostic, or prognostic evidence. If applicable, this section also lists resistance mutations with a corresponding note.

E. According to the KB, there is evidence of clinical significance for this biomarker in this tumor type based on information from FDA-approved therapy, clinical guidelines, or both. For more information on criteria for biomarkers included in this section, refer to *Cancer Mutations with Evidence of Clinical Significance* on page 16.

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 FDA-approved or investigational therapy. For more information on criteria for biomarkers included in this section, refer to *Cancer Mutations with Potential Clinical Significance* on page 17.

G. TMB is always listed in Cancer Mutations with Potential Clinical Significance section. Refer to *Tumor Mutational Burden* 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 16.



I. 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 *Companion Diagnostics QC* on page 30.

J. 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 (US) Package Insert (document # 200061832) for more information about the TSO Comprehensive intended use. Tumor type, Biomarker, and Therapy are from the Intended Use statement.

K. 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 to the *Companion Diagnostics Intended Uses Evaluated* on page 30.

- **Yes**—The sample was evaluated for this intended use. Specific results would be identified in the Companion Diagnostic 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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
chr10	43609942	GAGCTGTGCCGCACG	TCCAGC	RET	NP_066124.1:p.(Glu632_Thr636delinsSerSer)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
chr10	43609943	AGCTGTGCCGCACGGTGATCGCAGCC	TTCGACCG	RET	NP_066124.1:p.(Glu632_Ala640delinsValArgPro)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
chr10	43609950	CCGC	TCCGAAGCGT	RET	NP_066124.1:p.(Cys634_Arg635insProLys)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)

Chromosome	Position (hg19)	Reference Allele	Alternate Allele	Gene	Amino Acid Change
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)
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)

Appendix E Install a Knowledge Base

The TSO Comprehensive (US) analysis module requires an installed Knowledge Base (KB) to perform analysis. KBs are zip files 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 (US) analysis module. When updating a KB, the previously installed KB is removed during the installation process. Do not install a KB 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.

⚠ CAUTION

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

1. Download the desired KB (*.zip) 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 download it again from the portal.
3. Open TSO Comprehensive (US) analysis module on your instrument or the networked computer (local area network).
4. Sign in as an admin or a non-admin user with permission to edit module settings.
   For more information on TSO Comprehensive (US) analysis module user management, refer to *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.
5. Use the Tools menu to navigate to the Modules & Manifests screen.
6. Select **TSO Comp (US)**.
7. Select **Install New** under the Knowledge Base Version section.
   An installation wizard prompts you to browse to the location of the KB ZIP file.
8. Select the KB that was 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 (US) analysis module and that the KB is not corrupt. It is not possible to launch a new TSO Comprehensive analysis while installing the KB. After installation is complete, the new KB is listed on the Modules & Manifests screen. The KB name and version are also displayed on the Create Run, Requeue Analysis, and Edit Run screens.

Appendix F Cybersecurity

Antivirus or Antimalware Software

The following antivirus (AV) or antimalware (AM) software products are compatible with NOS and TSO Comprehensive (US) analysis module when configured following the instructions provided in the *NextSeq 550Dx Site Prep Guide (doc # 1000000009869)*:

- Windows Defender/Windows Security
- BitDefender
- CrowdStrike

For additional details regarding network, firewall, and storage configurations, contact Illumina Technical Support.

TSO Comprehensive Assay Security Certificate

The TSO Comprehensive (US) analysis module uses HTTPS to encrypt data connections to make sure that run data is private and secure. HTTPS is required for remote access of the instrument using a web browser from another machine in the same network. The TSO Comprehensive (US) analysis module requires the installation of a TSO Comprehensive security certificate in addition to the NextSeq 550Dx instrument TSO Comprehensive (US) analysis module security certificate.

NOTE If the TSO Comprehensive (US) analysis module Security Patch is installed on a NextSeq 550Dx instrument, then remote access from the customer-supplied PC via web browser using HTTPS to the NextSeq 550Dx Local Run Manager web portal is disabled.

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

1. Open TSO Comprehensive (US) analysis module on your instrument.
2. Use the Tools menu to navigate to the Modules & Manifests screen.
3. Select **TSO Comp (US) module**.
4. Download the TSO Comprehensive (US) 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

If the instrument name changed recently, or the instrument was moved to a new domain, you must regenerate the security certificate to regain access to the NextSeq 550Dx instrument and the TSO Comprehensive (US) analysis module. For instructions on how to regenerate the TSO Comprehensive (US) analysis module security certificate, refer to the *NextSeq 550Dx Site Prep Guide (doc # 1000000009869)*.

Revision History

Document	Date	Description of Change
Document # 200061834 v01	November 2024	• Updated Package Insert reference to appropriate document number • Updated software description
Document # 200061834 v00	October 2024	Initial release

Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

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

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

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