DRAGEN TSO 500 Analysis Software Release Notes

V2.6.1

For TruSight Oncology 500, TruSight Oncology 500 HRD, and TruSight Oncology 500 High-Throughput

January 15, 2025

Introduction

These Release Notes detail the key features and known limitations for the DRAGEN TSO500 v2.6.1 Analysis Software on the DRAGEN server.

This software is intended for use with the TruSight Oncology 500, TruSight Oncology 500 High-Throughput, and TruSight Oncology 500 HRD assays.

- Software Version: 2.6.1
- Docker Image ID: 00326573934d
- DRAGEN version inside of the TSO500 Docker Image: 3.10.17
- DRAGEN software version on the host DRAGEN server: 3.10.19

NEW FEATURES:

- Updated installer with multi-version DRAGEN support: DRAGEN TSO500 Analysis Software v2.6.1 can run on the same DRAGEN server with DRAGEN pipelines v4.3 or higher (e.g., DRAGEN Enrichment 4.3)

DEFECT REPAIRS:

- None

KNOWN ISSUES:

- If Sample Feature is labeled as an HRD sample, but HRD probes fail, the CNV output will be marked as "NA" in the CombinedVariantOutput.tsv. The CNV results can be recovered by removing the HRD label in the sample sheet. This issue occurs in previous versions with absolute copy number enabled (v2.5.0 and above).
- MetricsOutput file is not always generated when input BCL files are corrupt or missing. Users are recommended to check the Errors.tsv file for analysis status. ILMN Ref. A34561.

PRODUCT LIMITATIONS:

- Performance not verified using reads other than 2 x 101.
- The values in the Run Metrics section will be listed as 'NA' if the analysis was started from FASTQs or if the analysis was started from BCLs but the InterOp files are missing or corrupted.
- The TSO 500 RNA workflow is unstranded. Fusions or splice variants could involve antisense transcripts instead of the reported genes.
- A high number of chimeric reads due to poor quality RNA libraries can lead to false positive RNA fusions reported.

- TMB number may be inflated in samples with >5% supplementary (chimeric) alignments due to the larger number of false positive indels. ILMN Ref. 8507.
- Germline estimation that is used for TMB calculation uses the latest publicly available population data and is estimated to be representative of targeted population. The impact of rare germline mutations is expected to be limited for the TMB estimation.
- Germline estimation is difficult when tumor purity is > 85% causing expected variant allele frequency for somatic and germline variants to converge.
- Some regions are known to be difficult to sequence. One example region is the TERT promoter region. Although sequencing can occur at the TERT promoter region, this location might result in low coverage due to the GC rich content of the sequenced region. Another example region is the PMS2 gene which has high homology to pseudogenes and reads may not align properly. In general, the TSO 500 panel is designed to target unique regions, and the software accounts for background noise during small variant calling for each genomic position. This design is meant to prevent false positive calls. Analytical performance of the assay is evaluated panel-wide rather than for each gene or exon. However, due to these challenges certain regions covered in the product manifest are excluded from analysis due to high background noise. All excluded variants are identified in the VCF using a flag. This block list includes the following genes: HLA-A, HLA-B, HLA-C, KMT2B, KMT2C, KMT2D, chrY and positions with VAF > 1% occurred in six or more of the 60 baseline samples. The block list of excluded sites can be obtained on request from your local Illumina representative.
- Lower sensitivity and specificity may be seen in CNV amplifications and deletions with less than 20 probes and higher noise profiles. Contact your local Illumina representative for more details. The following genes are excluded from CNV calling due to high homology: HLA-A, HLA-B, HLA-C, KMT2B, KMT2C, KMT2D, HIST2H3A, HIST2H3C. These genes are excluded from CNV calling due to insufficient probe coverage (1 probe): DNAJB1, FANCF, FOXL2, HIST1H3A, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3F, HIST1H3G, HIST1H3H, HIST1H3I, HIST1H3J, HIST2H3D, TERC, and TERT (only covers promoter region); results for these genes are included in the VCF but are not included in the CombinedVariantOutput.tsv. ILMN Ref. 34500.
- Illumina Connected Annotations (formerly, Illumina Annotation Engine, Nirvana) may report incorrect HGVS c. and HGVS p. notation for small variants occurring in RefSeq transcripts that exhibit transcript sequences differing from the genomic reference (i.e., RNA-edits). Currently the HGVS c. error rate is 0.00527% and the HGVS p. error rate is 0.00737%.
- When a single nucleotide insertion introduces only a stop codon, it should be annotated as stop_gained, but instead Illumina Connected Annotations (formerly, Illumina Annotation Engine, Nirvana) annotates the event as a frameshift_variant. ILMN Ref. 33596.
- BRCA1 and BRCA2 large rearrangements (exon-level CNVs) with two segments that diverge equidistant from baseline in opposite directions in highly rearranged genomes would occasionally report a "GAIN" due to variation in the calculated distance from baseline. These samples are expected to have high genomic instability and will be filtered as “undetermined”.
- False negatives for BRCA1 and BRCA2 large rearrangements (exon-level CNVs) with a single or partial exon loss or gain and VAF lower than 61% are observed at higher rate than presented in product specification (sensitivity of 95% at VAF 50% or higher for fewer than 3 exons) due to the higher amount of noise associated with the smaller segment size. Pathogenic variants with single or partial exon CNVs are expected to have a prevalence of 0.17% in ovarian cancer

samples (Jones et al., Genes Chromosomes Cancer.2023;62:589–596). The current implementation was designed to reduce false positives and has shown to have a high gene-level specificity (100%) with internal testing. ILMN Ref. 28747

- Genomic Instability Score and BRCA large rearrangements (exon-level CNVs) have not been verified with input over 80ng of FFPE.
- GIS analysis has not been verified using libraries with UDP indexes.
- RNA DRAGEN mapping in lower quality samples has been found to have a high number of duplicates that are not marked, leading to SpliceGirl to incorrectly call RNA fusions and splice variants. ILMN Ref. 35248.
- A lower call rate of RNA fusions can occur due to germline variants near a breakpoint which penalizes the alignment score leading to lower number of supplementary alignments. This prevents supplementary alignments from being counted as supporting reads. This is an RNA DRAGEN mapper limitation. ILMN Ref. 32445.
- The estimates for tumor fraction and ploidy may be less reliable for samples with lower Genomic Instability Score as they will have fewer genome rearrangements.
- The contamination score threshold will fail approximately 1% of HRD samples due to the variant allele frequency (VAF) shifts of highly rearranged genomes and not true contamination of foreign human DNA. Visual investigation of VAFs across the genome can be performed to determine if a shift of VAFs is due to true contamination.
- DRAGEN small variant caller performs realignment of reads to reconstructed haplotypes, if a realigned read does not have the same length of CIGAR string as the original alignment a sample will fail small variant calling. This is a very rare occurrence and resequencing of the library has been found to remove the read causing this error. ILMN Ref. 34810.
- The number of transcripts per million (TPM) in the .quant.genes.sf file was not verified and is for information only.
- Several bioinformatics features have *beta* status. Beta features have not been verified by Illumina due to limited access to samples *or* lack of an appropriate orthogonal method to perform testing, and the use of *in silico* testing alone is not sufficient for verification purposes. Beta features are only available with the TSO 500 HRD kit and include:
- Tumor fraction
- Ploidy
- Absolute copy numbers
- Gene-level loss of heterozygosity (LOH) events

Release History

Revision	Release Reference	Originator	Description of Change
00	CN 1117527	Svetlana Bureeva	Initial Release