

DRAGEN TSO500 ctDNA Analysis Software

Customer Release Notes

v2.6.3

For TruSight Oncology 500 ctDNA Assay

November 13, 2025

Introduction

These Release Notes detail the key changes to software components for the DRAGEN TSO500 ctDNA v2.6.3 Analysis Software. For full details, please consult the DRAGEN TSO 500 ctDNA Analysis Software v2.6.3 User Guide available on the support website.

This software is intended for use with the TruSight Oncology 500 ctDNA and TruSight Oncology 500 ctDNA v2 assays.

DRAGEN TSO 500 ctDNA v2.6.3 Analysis Software is available:

- On DRAGEN server
- On Illumina Connected Analytics (ICA)
- As NovaSeq 6000Dx Analysis Application

These Release Notes also cover features of BaseSpace Run Planning tool related to run set-up and sample sheet generation for DRAGEN TSO 500 ctDNA Analysis Software.

This document describes features and limitations for all abovementioned platforms. If an item is specific to a software platform, it will be specified using a tag: "ICA," "Server," "NovaSeq 6000Dx app," "BaseSpace Run Planning Tool."

NEW FEATURES:

The features listed here include all changes since v2.6.1 (the most recent version available on all deployments). If a feature was introduced in an earlier version (e.g. v2.6.2 or v2.6.1.8) it is annotated accordingly. All other features are new as of v2.6.3.

- The software now supports data from NextSeq 2000 (P4 flow cells). Additionally, the software now supports the following additional flow cells: NovaSeq 6000/ 6000Dx S1, NovaSeq X 25B.
- The software now supports running TSO 500 ctDNA v2 assay with Illumina UMI DNA/RNA UD v3 indexes.
- DRAGEN version was upgraded to 3.11.2. Installation on a standalone DRAGEN server requires DRAGEN version 3.11.2 or above.
- The software writes values for fields in BCL Convert Settings section to the intermediate sample sheet based on the Library Prep kit used in a run (determined from index sequence length). The overwritten fields are: AdapterRead1, AdapterRead2, MaskShortReads, AdapterBehavior, MinimumTrimmedReadLength
- LibraryPrepKits field now accepts multiple values (separated by semicolons), but only one value can be a supported TSO 500 ctDNA kit. (e.g. "TSO500_ctDNA; TSO500_v2" is allowed, but "TSO500_ctDNA; TSO500_ctDNA_v2_UDPv3" is not allowed)
- Software now accepts sample sheets where only a subset of sample IDs from BCL Convert Data section are listed in TSO500L_Data (or Cloud_TSO500L_Data) section. When starting analysis from run folder, the software will only retain FASTQ files for samples listed in the TSO500L_Data (or Cloud_TSO500L_Data) section.
- OpenSSL library in resource bundle updated to 1:1.1.1k-14

- When starting from FASTQ, Instrument Type is now determined from FASTQ headers and must match across all FASTQ files. If no Instrument Type can be determined, software checks input sample sheet.
- Combined Variant Output no longer includes RTA version in Sequencing Settings section.
- Combined Variant Output now includes the following information in Sequencing Settings section: Instrument Type (determined from run folder, FASTQ headers, or input sample sheet), Instrument Software Version (when available from run folder), Instrument Platform (when available from input sample sheet).
- Metrics Output: For run folders generated from NextSeq 2000, PCT_PF_READS is excluded from the Run QC Metrics section.
- (Server) Time zone used by pipeline now matches server local time.
- (Server) When user specifies output folder in the bash wrapper, software checks that it has the necessary permissions, that the folder exists and is empty. If not, it exits with an error message.
- (Server, as of v2.6.2) Updated installer with multi-version DRAGEN support: DRAGEN TSO500 ctDNA Analysis Software v2.6.3 can run on the same DRAGEN server with DRAGEN pipelines v4.3.6 or higher (e.g., DRAGEN Enrichment 4.3.6) Note: For co-installation compatibility with other versions of DRAGEN TSO 500 ctDNA, see user guide.
- (ICA, as of v2.6.1.8) Pipeline update to run on either F1 or F2 nodes, depending on availability.
- (ICA) Auto-launch of DRAGEN TSO 500 ctDNA v2.6.3 Analysis Software on ICA when starting the analysis with FASTQ files. Selecting TRUE for "Starts from FASTQ" in BSSH Run Planner will enable auto-launch of DRAGEN BCL Convert 3.10.9 before auto-launching DRAGEN TSO 500 v2.6.3 Analysis Software on ICA.
- (ICA) Auto-launch of multiple pipelines now supported to enable fully automated workflow when mixing different assays on one flow cell.
- (ICA) BCL Convert updated to a version that does not require FPGA nodes for processing, reducing queue time for demultiplexing.
- (ICA) BCL Convert runs on each lane in parallel, reducing processing time required for demultiplexing.
- (ICA) Additional details added to log file (command.Error in workdir/) when pipeline encounters file not found error.
- (BaseSpace Run Planning Tool) Support for run planning and sample sheet generation for TSO 500 ctDNA v2 libraries on NovaSeq 6000/ 6000Dx, NovaSeq X, and NextSeq 2000.

DEFECT REPAIRS:

- Fixed an issue where MetricsOutput file was not always generated when input BCL files are corrupt or missing. ILMN Ref. A34561.
- Fixed an issue where software exits abruptly when calling Fusions on No-Template Control (NTC) samples. ILMN Ref. A34412

- Fixed a rare issue where DRAGEN small variant caller failed while realigning reads to haplotypes, resulting in no results output and requiring resequencing as a workaround. ILMN Ref. A34810
- Fixed an issue where instrument software version was not output in Combined Variant Output. ILMN Ref. A33387
- Fixed an issue where software errors out erroneously when sample sheet validator detects invalid index sequences but analysis is starting from FASTQ. ILM Ref. A39270
- (Server) Fixed an issue where software was unable to run due to CPU requirements for DRAGEN servers. ILMN Ref. A40156
- (Server) Fixed an issue where the installer was unable to install DRAGEN when DRAGEN v3.10.9 is already installed. ILMN Ref. A39987
- (ICA) Fixed an issue where runs failed sporadically on ICA. ILMN Ref. A35888
- (ICA) Fixed an issue where ICA runs fail at Reporting step with unrecognized character. ILMN Ref. A36491

KNOWN ISSUES:

- There are minor differences in outputs of exon_cov_report and gene_cov_report from DRAGEN TSO 500 Analysis Software on ICA and DRAGEN server/NovaSeq 6000Dx Analysis Application
- The sample sheet should not have blank rows between samples in the [Data] section, this may cause a run failure.
- Performance not verified using reads other than 2 x 151, paired end, dual index.
- The software does not notify the user when InterOp files for RunQC are missing or corrupted.
- Analysis fails when starting from V1 sample sheets due to missing adapter sequences in V1 sample sheet template. Users are recommended to start with V2 sample sheet template or add adapter sequences manually.
- Pipeline does not exit early and continues to the next DragenCaller step due to TSO500 ctDNA FASTQ validation failure if Fastq_list.csv is missing.
- High chimeric read count results in incorrect TMB calculation.
- In the V2 CNV cutoff bed file, gene "MYCL" should be listed instead of "MCYL1".
- Variant consequences are not assigned consistently for co-occurring variants when stop_gained is introduced. ILMN Ref. A33388
- (Server) Moving or modifying files during the analysis may cause the analysis to fail or provide incorrect results.
- (Server) Using control-c during a running analysis may cause an FPGA error. To recover from an FPGA error, shut down and restart the server.
- (NovaSeq 6000Dx app) When launching analysis after installing another NovaSeq 6000Dx app and its dependent version of DRAGEN (that does not support multi-version installation), the software may sporadically be unable to run. A workaround is provided in the user guide. ILMN Ref. ILMN Ref. A37833.
- (NovaSeq 6000Dx app) The application will launch an analysis when the storage on the server is less than 4TB, which may potentially run out of space. ILMN Ref. A28756

- (NovaSeq 6000Dx app) The "Next" button in the application is not available while trying to create a run using Import Run with a sample sheet that contains the Sample_ID in the [TSO500L_Data] section but not in the [BCLConvert_Data] section. ILMN Ref. A24914
- (NovaSeq 6000Dx app) When using a Samplesheet where Sample_ID column is missing from BCLConvert_Data or [TSO500L_Data] section for Import Run, error message is displayed, and there's a typo "sample_idin" (missing a space). ILMN Ref. A24998
- (NovaSeq 6000Dx app) Requeue analysis with no changes throws an error. It works when the second option "Edit run settings and requeue analysis" is selected. ILMN Ref. A32183
- (NovaSeq 6000Dx app) On the Results page, the UI displays a section header titled Sample ID, but no results are displayed in the table below. ILMN Ref. A25859.
- (NovaSeq 6000Dx app) Analysis Run Results not displayed in run details for runs started by users with the role Sequencer Operator User. ILMN Ref. E10169

PRODUCT LIMITATIONS:

- The sample sheet must be configured as described in the User Guide or by using BaseSpace Run Planning tool.
- Sample sheets generated for auto-launch on ICA are not compatible and cannot be reused without changes for DRAGEN TSO500 ctDNA Analysis Software on a Local DRAGEN server, and vice versa.
- (ICA) ICA run time depends on ICA instance availability, it will be affected by region and traffic
- (ICA) When storage is not selected as "XLarge" for NovaSeqX 25B flow cells and at least "Large" for every other flow cells the run will fail a disk space check.
- (ICA) Added validation for the storage size selection generates an error if "Small" or "Medium" values are selected ("Large" is required as a minimum) but the error message appears with a delay.
- 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.
- Germline estimation 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.
- The Illumina Annotation Engine (aka 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%.
- The CNV caller has slightly higher noise for sample types that are not included in the baseline used for normalization (e.g., cell lines). The baseline samples consist of mostly healthy donor clinical samples and SeraCare-contrived samples.
- MSAF output has had limited testing and needs to be used with caution. Updates to the small variant calling have led to an increased MSAF in samples with higher DNA input.
- Variant reporting is limited by a manifest file and a block list file. The manifest file excludes regions where the probe set does not effectively capture targets, and the block list file excludes specific positions from variant calling. TSO 500 probes target at least 97% of the CDS of 474 genes. Please contact your local Illumina representative for more information if needed.

Release History

Revision	Release Reference	Originator	Description of Change
00	CN 1130369	Cordelia Ziraldo	Initial Release