DRAGEN TSO500 ctDNA Analysis Software

Customer Release Notes

V2.1

For TruSight Oncology 500 ctDNA Assay

December 15, 2023

Introduction

These Release Notes detail the key changes to software components for the DRAGEN TSO500 ctDNA v2.1 Analysis Software since the package containing DRAGEN TSO500 ctDNA v1.2 Analysis Software.

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

- Software Version: 2.1.1
- DRAGEN software version 3.10.9

The software package includes:

- dragen_tso500_ctdna_2.1.tar – a tar file of the DRAGEN_TSO500_ctDNA_v2.1 docker image
- uninstall_DRAGEN_TruSight_Oncology_500_ctDNA -2.1.0.sh – a script for uninstalling DRAGEN_TSO500_ctDNA_v2.1
- resources/ - a directory containing all resources files necessary for DRAGEN_TSO500_ctDNA_v2.1
- DRAGEN installer:
    - dragen-3.10.9-8.el7.x86_64.run–theDRAGENinstallerforCentOS7system
    - dragen-3.10.9-8.el8.x86_64.run–theDRAGENinstallerforOracle8system
- test_DRAGEN_TruSight_Oncology_500_ctDNA-2.1.0.sh - a self-test script for verifying the software installation
- install_DRAGEN_TruSight_Oncology_500_ctDNA-2.1.0.run – The script used to install TSO500 ctDNA based on the contents listed here
- sudo chmod +x /staging/install_DRAGEN_TruSight_Oncology_500_ctDNA-2.1.0.run to update the permissions on the run script
- sudo TMPDIR=/staging /staging/install_DRAGEN_TruSight_Oncology_500_ctDNA -2.1.0.run install script

NEW FEATURES:

- Describe new features by feature type (e.g., Inputs, Reporting, Outputs, Filtering). If there are none, simply list "None"
- The following components were replaced with a DRAGEN version from a non-DRAGEN version for improved speed and accuracy:
    - MSI module
    - CNV caller
    - TMB module
    - Small Variant Caller
    - Sample QC module
- Performance improvements:

  - Improved MSI specificity
  - Enhanced bTMB accuracy due to improved filtering of CH and germline variants
  - Improved specificity for small variant calling
  - Ability to call complex variants panel-wide
  - Ability to call insertions and deletions > 25 bp
  - Improved Contamination QC to better handle samples with highly rearranged genomes
- Fusion directionality is now reported in the combined variant output
- The Illumina Annotation Engine (Nirvana) was updated to version 3.2.6
- Spoiler was updated for contamination calling
- The software now accepts FASTQ files generated by the stand-alone BCLconvert software
- The installer was updated to be compatible with Oracle Linux 8 and CentOS 7
- The software now provides a Metrics Output per sample as well as for the full run
- The Analysis Output has been updated:
  - Now it generates an output folder in the specified location with the folder name "DRAGEN_TSO500_ctDNA_ Analysis_YYYMMDD-HHMMSS"
    - Within the analysis folder, each analysis step generates a subfolder within the Logs_ Intermediates folder.
  - Inputs to the running docker container are mapped from native locations on the server to the following locations in the container:

Input	Running Docker Container Location
Run folder	/opt/illumina/run-folder Sample
Sample sheet	/opt/illumina/SampleSheet.csv
FASTQ folder	/opt/illumina/fastq-folder
Resources	/opt/illumina/resources
Analysis output folder	/opt/illumina/analysis-folder

- The pipeline was updated to reduce the time to failure in case of sample sheet errors. This was accomplished by running validation ahead of all other steps and allowing the step to be executed on a more widely available node. The current 'time to failure' now corresponds directly to the size of the input run or FASTQ folder, as this must first be copied into a scratch location to support SampleSheetValidation.

FIXED ISSUES:

- Illumina Annotation Engine 3.2.6 (aka Nirvana) includes the following enhancements and bug fixes:
  - Added genes and transcripts from the NCBI Homo sapiens Updated Annotation Release 105.20201022 to provide the latest RefSeq content for GRCh37

- Reduced the HGVS c. error rate by 54% and HGVS p. error rate by 20%. Currently the HGVS c. error rate is 0.00527% and the HGVS p. error rate is 0.00737%.
- Fixed issues related to incorrect CDS coordinates in some edge cases
- Improved detection of frameshifts when variants partially overlap the coding sequence

KNOWN ISSUES:

- Installation of the software can result in a Docker error. To fix this, run the following command and then rerun the installer:

  yum remove -y docker-ce docker-ce-cli containerd.io && yum clean all yum remove docker-buildx- plugin
- Moving or modifying files during the analysis may cause the analysis to fail or provide incorrect results.
- Using control-c during a running analysis may cause an FPGA error. To recover from an FPGA error, shut down and restart the server.
- 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 metrics output step module shows no error message when input file is missing.
- FastqGeneration issue: Missing .bcl files can cause FastqGeneration failure, but pipeline does not generate a MetricsOutput.tsv file with failed the steps.
- The software does not notify the user when InterOp files for RunQC are missing or corrupted.
- For runs with samples with extreme copy number gains (e.g. fold change > 50, corresponding to ~250 copies when tumor fraction is ~40%) in a particular region or contrived samples, the runtime may take significantly longer than 20 hours.
- Some contrived samples such as SeraCare Complete Mutation Mix, which have multiple structural variants (SVs) and high conversion efficiencies, could generate a high number of chimeric reads and high number of candidate SVs. Occasionally, the SV caller may filter some of the reads and lead to occasionally missing fusions. In such cases downsampling the FASTQs can help recover those fusion calls. Contact your local support team for additional details and a workaround.

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 are not compatible and cannot be reused without changes for DRAGEN TSO500 ctDNA Analysis Software v2.1.1 on a Local DRAGEN server, and vice versa.
- 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 (eg., cell lines). The baseline samples consist of mostly healthy donor clinical samples and SeraCare-contrived samples.

Release History

Revision	Release Reference	Originator	Description of Change
00	1075803	Darryl Leon	Initial Release
01	1075803	Maria Jarama	Update to address Nirvana bg fix
02	1075803	Maria Jarama	Update to address installer issue
03	1096424	Svetlana Bureeva	Added support for the new assay version