TSO500 RUO Software Release Notes

V2.0.1

For TruSight Oncology 500 Assay

September 11, 2020

Introduction

The release notes detail the key features and known limitations for the TSO500 v2.0.1 local app and local run manager module.

Local App

This software is intended for use with the TruSight Oncology 500 Assay.

- Software Version: 2.0.1

The software package includes:

- trusight-oncology-500-ruo-2.0.1.zip

Local Run Manager Module

This software is intended for use with the TruSight Oncology 500 Assay.

- Software Version: 2.0.1

The software package includes:

- trusight-oncology-500-ruo-2.0.1.zip

NEW FEATURES:

- None

DEFECT REPAIRS:

- Fixed Combined Variant Output File Truncation Issue related to handling of reference minor allele variants

KNOWN ISSUES:

- Indel Realignment and Read Stitching algorithm can produce output stitched BAM files that vary by as many as 5 reads due to a reproducibility issue with sorting order, which can lead to variation in DNA QC Metrics less than .01%
- Across multiple analyses on the same compute environment, the phased variant algorithm can produce different variant calls for variants with equal levels of supporting evidence within EGFR exon 19 region (seen in less than 1/100 samples)
- Local app may use additional available hardware/compute resources, recommendation is to not run multiple local app instances on a single node

- Performance not verified using read lengths other than 2 x 101
- The NextSeq instrument software memory consumption may prevent the TSO500 Local Run Manager module from starting. An error message in the module logs beginning “Failed to start the virtual machine…” indicates this issue (located in the directory “{Run_ID} \Analysis_N \YYYYMMDD_HHMMSS \Module_Logs”). After encountering the issue, please perform the following steps.
    - Use the “Exit to Windows” command to shut down the instrument software.
    - Restart the NextSeq Control Software.
    - Re-queue the run.

PRODUCT LIMITATIONS:

- Unmapped long insertions are not likely to occur on shorter indels. This is because there is sufficient reference-matching sequence in the reads. Product claims only indels up to 25 base pairs.
- Complex variants are specifically output only for a specific region of the EGFR gene, component and phased variants would both be contained in the output
- Incorrect calculation of variant allele frequency can occur in variants near the start and end of genomic reads, but variation in read start and end positions in an enrichment assay is sufficient to make incorrect variant allele frequency in output variants a low-probability situation.
- Germline estimation using high tumor purity (>70%) can impact estimation, due to somatic and germline variants appearing with similar variant allele frequency.
- Germline estimation uses latest publicly available population data and estimated to be representative of targeted population, the impact of rare germline mutations is expected to be limited
- Poor quality wild type reads may align as chimeric and be miscalled during RNA analysis
- Manta will not call fusions where both breakpoints map to the same gene transcript: FIP1L1-PDGFRA (when both breakpoints overlap Ensembl transcript ENST00000507166) and GOPC-ROS1 (when both breakpoints overlap Ensembl transcript ENST00000467125).
- Manta may not always call fusions in the following situations, which have been exclusively observed in synthetic commercial controls:
    - Multiple fusion breakpoints from a single fusion gene pair with breakpoints within approximately 150 base pairs of each other (observed with ETV6-ABL1 and ETV6-NTRK3 fusions).
    - Multiple fusions from two different gene pairs with breakpoints within approximately 150 base pairs of each other (observed with IRF2BP2-NTRK1, TFG-NTRK1, SQSTM1-NTRK1 fusions).
    - Breakpoint(s) are located in region(s) with high homology (observed with fusions with breakpoints on SEPT14 exon 10).

Release History

Version	ER#	Author	Description of Change
00	DIR Workflow	Trey Howard	Initial Release