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TSO500 Local App Software
Release Notes

V2.1.0

For TruSight Oncology 500 Assay

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Introduction

These Release Notes detail the key features and known limitations to software components for the Local TSO500 App v2.1.0

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

- Software Version: 2.1.0.60

The software package includes:

- trusight-oncology-500-ruo-2.1.0.60.zip
    - o md5sum.txt
    - o resources
    - o trusight-oncology-500-ruo-dockerimage-ruo-2.1.0.60.tar
    - o trusight-oncology-500-ruo.img
    - o TruSight_Oncology_500_RUO.sh

NEW FEATURES:

- Support for NovaSeq 6000
    - o Unique Dual Indices
    - o Multi-node analysis (Scatter/Gather)
    - o Updated Run QC Metrics
- Additional Indices for NextSeq 500/550, 550Dx (RUO Mode)

DEFECT REPAIRS:

- Updated Read Stitching and Indel Realignment Component (Gemini) to create reproducible BAM file and VCF results
- Updated software to resolve rare instances of truncated combined variant output files

KNOWN ISSUES:

- Local app may use additional available hardware/compute resources, recommendation is to not run multiple local app instances on a single node
- FASTQ Generation can produce empty FASTQ files in rare instances of NextSeq tile drop, which causes software failures. Removal of the empty FASTQ files allow for continuation of processing
- Performance not verified using read lengths other than 2 x 101
- FASTQ Generation is not support with read lengths other than 2 x 101 or 2 x 151
- When using NovaSeq XP workflow, sample sheets require lane information and may provide unclear error messages when lane information is excluded

- An error will occur when trying to run more than 96 samples on a single node
- Rare instances of low multiplexed runs (3 DNA samples in a run) cause delayed results when performing read collapsing
- Combined Variant Output contains mislabeled fields in the Splice Variants Section for Splice Supporting Reads and Reference Reads. The values are correct based on the VCF but the headers are switched.
- Gathering output requires all samples scattered to be gathered at once, gathering a subset of results is not supported.

PRODUCT LIMITATIONS:

- Unmapped long insertions are not likely to occur on shorter indels 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
- When filtering fusion variant candidates, strand consideration is not used in annotation, which allows contiguous sequences to be intragenic, when they are on different strands
- 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	Kyle Cutler	Initial Release