TruSight Tumor 170 RUO Local App

Customer Release Notes

V2.0.0.39

For TruSight Tumor 170 Reagents

Introduction

These Release Notes detail the key features and known limitations for this version of the TruSight Tumor 170 local analysis application. This release delivers a number of new features and major pipeline changes: we recommend that customers revalidate their informatics workflow prior to adoption of this version.

I. TruSight Tumor 170 Local App Version 2.0.0.39

NEW FEATURES:

1. Pipeline improvements.
    a. Demultiplexing
        i. Removed DNA down-sampling.
        ii. Implemented an alternative adapter-trimming method.
    b. DNA Alignment
        i. Aligner changed from Isaac to BWA-MEM.
        ii. Implemented an alternative method for duplicate marking.
    c. Small Variant Calling
        i. Improved indel realignment.
        ii. Improved variant calling sensitivity.
        iii. Addition of intronic sites by expanding manifest by +/-2bp for splice sites.
    d. Complex Variant Calling
        i. Pipeline now detects Complex Variants (insertion/deletion combinations) in EGFR.
    e. RNA
        i. Improved duplicate marking logic.
        ii. Updated STAR to improve handling of overlapping reads.
        iii. Improved variant calling performance.
        iv. Alternative method for adapter trimming.
        v. Change down-sample limit from 300 million reads to 30 million reads.
        vi. Fusions.csv now indicates whether directionality could be established for each fusion.
    f. General
        i. Annotated variant files are now output in JSON format. VCF files are still produced by the pipeline, but they do not contain annotations.
        ii. Nine (9) additional QC metrics added for DNA libraries; five (5) additional QC metrics added for RNA libraries
        iii. Updated metric calculation for Median_Insert_Size.
        iv. Introduction of "Pair ID" column to sample sheet to support combined variant reporting (see below).

2. New Reports
    a. A new MetricsOutput file combines QC outputs for all samples in an analysis into a single TSV file:
        i. Run-Level QC Metrics
        ii. Pipeline completion status for individual samples.
        iii. DNA + RNA QC metrics for individual samples.

b. The new Combined Variant Report file is designed to facilitate reporting on DNA and RNA libraries extracted from the same sample (identified by "Pair ID" in the sample sheet). For selected DNA + RNA sample pairs, this report merges the content below.
    i. Small variants (passing variants only)
    ii. EGFR complex variants (passing variants only)
    iii. MSI results
    iv. Copy number variants
    v. Splice Variants (high confidence only)
    vi. Fusions (high confidence only)

3. Microsatellite Instability
    a. An algorithm for calculating Microsatellite Instability score for DNA samples has been incorporated into TST170.

DEFECT REPAIRS:

- Variant calling failure that occurred when a variant was detected at the 49th position from the end of a chromosome.
- The annotations contained in the PublishedFusions.csv file had become dated. This file has been removed.
- Out of memory failures associated with the Isaac aligner have been resolved by replacing this with BWA-MEM.
- An issue which could cause the "Percent on Target Reads" RNA QC metric to be miscalculated by ~0.01% has been addressed by RNA pipeline updates.
- An issue in regions of low coverage which could cause the number of DEDUP reads in splice variant calculation to be greater than DUP read count.
- A version update of the Illumina Annotation Engine will permit analysis on machines with processors without support for AVX instructions.
- The Combined Variant Report no longer incorrectly labels sections as "Error" when they should be labeled as "N/A".
- The FusionFilter.csv intermediate file has been added back to the Logs_Intermediates/RNA_IntermediateFiles/FusionCalling output folder.
- Copy Number Variant Calling Log Files have been moved from the Logs_Intermediates/DNA_IntermediateFiles/CNV output folder to a new folder: Logs_Intermediates/Logs/DNA/CNV.

KNOWN ISSUES:

1. Splice variant coordinates may differ from standard VCF format recommendations. Exon and intron annotations should not be affected.
2. Fusions in the following cases will be called, but may not pass fusions score cutoff:
    - Breakpoints near repetitive genomic regions
    - Contig being misaligned as an intragenic sequence
3. Fusions in the following cases will have a decreased chance of being called:

- Loci with a high volume of sequencing errors
- Loci enriched for short fragments
- Loci with unique reads marked as duplicates

4. Fusions in the following cases may be called incorrectly:
   - Fusions of two genes from the same family
   - Fusions of two genes highly homologous to sequences in unassigned chromosomes
5. Certain scenarios cause inconsistencies in the labeling of the Combined Variant Report.
   - "N/A" fields labeled "Not Available".
     1. Example:
        - Report: CombinedVariantReport.tsv; Section: Splice Variant; Column: Affected Exon
6. The Allele Frequency column in the Combined Variant Report does not round to the thousandths place.
   - Example: The stated value is displayed as 0.0607. This should have been rounded and reported as 0.061.
7. When analysis fails demultiplexing, the individual variant sections within the Combined Variant Report are displaying 'Error' instead of 'N/A'.
   - Error should only be displayed when individual components are their direct dependents have a failure.