TruSight Tumor 170 RUO Local App

Customer Release Notes

V2.0.1.21

For TruSight Tumor 170 Reagents

Introduction

These release notes detail the improvements and known issues for this version of the TruSight Tumor 170 local analysis application. This release includes an update to the variant annotation pipeline component: we recommend that customers revalidate their informatics workflow prior to adoption of this version.

I. TruSight Tumor 170 Local App Version 2.0.1.21

DEFECT REPAIRS:

- Illumina Annotation Engine (aka Nirvana) updated to address over 99% of known situations where the software may report incorrect C-Dot and P-Dot notation values for DNA variants on affected RefSeq transcripts (see Known Issues below).

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
    - 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).
    - Fusions in the following situations, which have been exclusively observed in synthetic commercial controls:
        1. Multiple fusion breakpoints from a single fusion gene pair with breakpoints within approximately 150 base pairs of each other (observed with ETV6-ABL1 in the Seraseq Myeloid Fusion RNA Mix and ETV6-NTRK3 fusions in the SeraCare NTRK Fusion sample).
        2. Multiple fusions from two different gene pairs with breakpoints within approximately 150 base pairs of each other (observed with IRF2BP2-NTRK1, TFG-NTRK1, SQSTM1-NTRK fusions in the SeraSeq RNA fusion V4).
        3. Breakpoint(s) are located in region(s) with high homology (fusions with breakpoints on SEPT14 exon 10 in the Seracare RNA mix).”
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. The Illumina Annotation Engine may report incorrect protein (P-Dot) and transcript (C-Dot) changes in HGVS nomenclature for small variants located on a RefSeq transcript where an RNA-edit has occurred. Most known variants on these transcripts are unaffected. A list of affected Canonical RefSeq transcripts and Cosmic Variants from those transcripts can be found below. A full explanation of this product limitation can be found in PQN2020-1090.

    Affected Canonical RefSeq Transcripts [1]

Transcript ID	Gene Symbol
NM_002467.4	MYC
NM_004119.2	FLT3
NM_198291.2	SRC
NM_021960.4	MCL1

Affected Cosmic Variants from Canonical RefSeq Transcripts

The list of affected variants is based on an analysis of COSMIC database version 92 variants located along the Canonical RefSeq Transcripts listed above [2]. New variants are regularly submitted to COSMIC, and this list of affected variants may change over time.

Chr:Position	REF*	ALT**	Gene Symbol	Transcript ID	COSMIC_ID
chr1:150548890	A	ATCTA	MCL1	NM_021960.4	COSV57189597
chr8:128748839	GC	G	MYC	NM_002467.4	COSV104388447
chr8:128748840	C	A	MYC	NM_002467.4	COSV104388806
chr8:128748840	C	G	MYC	NM_002467.4	COSV104388204
chr8:128748841	T	C	MYC	NM_002467.4	COSV104388663
chr13:28608094	C	CACTTTTCCAAAAGCACCTGATCCTAGTACCTTCCCAA ACTCTAAATTTTCTCTTGGAAACTCCCATTTGAGATCA TATTCATATTCGTTCATC	FLT3	NM_004119.2	COSV54069050
chr13:28608124	C	CTTCCCAAACTCTACTGTTGCGTTCATCACTTTTCCAA AAGCACCTGATCCTAGTACC	FLT3	NM_004119.2	COSV54044227
chr13:28608129	C	CAAACTCAAAAGCACCTGATCCTAGTACCTTCCC	FLT3	NM_004119.2	COSV54054381
chr13:28608129	C	CAAACTCTAAATTTTCTCTTGGAAACTCCCATTATCCT AGTACCTTCCC	FLT3	NM_004119.2	COSV54043729
chr13:28608129	C	CAAACTCTAAATTTTCTCTTGGAAACTCCCATTTTCCA AAAGCACCTGATCCTAGTACCTTCCC	FLT3	NM_004119.2	COSV54075746
chr20:36030939	G	GTGGCC	SRC	NM_198291.2	COSV99050886

*Reference base(s)

**Alternate base(s)

[1] TST170 uses the Canonical RefSeq transcript when annotating variants passed into the Combined Variant Report file. The Illumina Annotation Engine selects canonical transcripts based on the following rules…

- Order all overlapping transcripts by coding sequence length.
- Pick the longest transcript that has an associated Locus Reference Genome (LRG) sequence.
- If no LRGs exist for the set of transcripts, pick the longest transcript that is coding.
- If there is a tie, pick the transcript with the smaller accession id number.

[2] Released 27 August 2020.