Local Run Manager TruSight Oncology 500 Analysis Module Release Notes

V2.4.0

For TruSight Oncology 500 Assay

October 30, 2024

Introduction

These Release Notes detail the key features and known limitations for the Local Run Manager TruSight Oncology 500 Analysis Module v2.4.0. This software is intended for use with the TruSight Oncology 500 Assay on NextSeq 500, NextSeq 550 and NextSeq 550Dx in RUO mode.

NEW FEATURES:

- The version is compatible with NextSeq Control Software v4.2.0 and Local Run Manager v4.0.0.
- The TSO 500 pipeline is updated to v2.2.1.
- The TSO 500 blocklist is updated to remove PMS2CL pseudogene.
- The VM Manager is updated to v4.2.0.
- LRM module installer is digitally signed with Illumina as the publisher.
- LRM Dashboard now shows the analysis job status per step during analysis.
- The LRM module resource bundle reads from Drive D.
- Cromwell workflow logs are now saved to the Logs_Intermediates folder.

DEFECT REPAIRS:

- Analysis that used to hang indefinitely due to hardware issues now fails immediately
- Illumina Connected Annotations (formerly, Illumina Annotation Engine, Nirvana) is updated to v3.2.7. This includes the following enhancements and bug fixes:
    - RefSeq content version 105.2020102
    - 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:

- Rare instances of low multiplexed runs (3 DNA samples in a run) cause delayed results when performing read collapsing.
- 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.
- In rare circumstances, the Run QC section of the Metrics Output file may fail to populate. Please review the RunQCMetrics.json file to troubleshoot the run.
- The descriptions for the "AD" and "DP" fields in the Splice VCF header are reversed.
- TSO 500 analysis will fail due to hardware issues reported by Windows Hyper-V. Requeue the analysis. If the problem persists, please contact techsupport@illumina.com as triple fault error may be caused by CPU overheating or other hardware errors.

PRODUCT LIMITATIONS:

- Performance not verified using read lengths other than 2 x 101.
- 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, constituent variants 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).
- Software may include small variants outside, but near, manifest regions in samples where small variant candidates partially overlapping manifest boundaries are evaluated.

- The Illumina Connected Annotations (formerly, Illumina Annotation Engine, 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%.

Release History

Revision	Release Reference	Originator	Description of Change
00	CN 1114469	Svetlana Bureeva	Initial Release