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TECHNICAL NOTE

Automating the TruSight™ Oncology 500 v2 workflow with the Hamilton NGS STARlet liquid handler

Streamlined, consistent library preparation with an optimized automated protocol

Optimized automated method

Optimized Illumina Partner Network Method ready to deploy immediately

Efficient library preparation

Streamlined workflow with minimal touchpoints, saves time and reduces opportunities for errors

Concordant performance

High-quality, consistent library generation results compared to manual preparation

Introduction

Comprehensive genomic profiling (CGP) has emerged as a critical tool in research laboratories, offering broad genomic assessment of multiple cancer types.[1] By simultaneously evaluating hundreds of genes for single nucleotide variants, copy number variations, gene fusions, and other genomic alterations, CGP can provide a holistic view of the molecular drivers of disease.

Illumina offers TruSight Oncology 500 v2, which enables reliable, large-scale sequencing through an efficient hybridization enrichment workflow and fast time to results.[2] Designed for scalability, it supports flexible batching and automated workflows across multiple Illumina sequencing platforms. TruSight Oncology 500 v2 delivers fast, high-accuracy variant calling, enabling CGP efficiency and accessibility for cancer research.

This technical note presents an Illumina Partner Network Method for preparing the TruSight Oncology 500 v2 assay on the Hamilton NGS STARlet liquid handler as part of a streamlined, comprehensive workflow (Figure 1). The method gives users the option to run the library enrichment overnight, further reducing turnaround times compared to the manual workflow. Results demonstrate that the automated workflow generates consistent libraries that yield high-quality data with less hands-on time and fewer opportunities for errors compared to the manual workflow.

Methods

Samples

For these validation studies, a mixture of control RNA and DNA samples were used. Samples included TruSight Oncology DNA Control (Illumina, Catalog no. 20065041), TruSight Oncology RNA Control (Illumina, Catalog no. 20065042), Mimix Structural Multiplex gDNA Reference Standard (Horizon Discovery, Catalog no. HD753), and Universal Human Reference RNA (Agilent, Catalog no. 740000).

Streamline library preparation with automation

The automated protocol for preparing TruSight Oncology 500 v2 libraries on the Hamilton NGS STARlet includes four modules: cDNA synthesis, library prep, enrichment, and normalization. These modules can be run with minimal touchpoints with safe stopping points throughout (Figure 2). The method can process 4–24 samples in a single batch with variable combinations of RNA and DNA samples, enabling an efficient workflow that can prepare sequence-ready libraries in < 2 days with minimal hands-on time. Illumina DNA/RNA UD Indexes v3 (Illumina, Catalog no. 10141196, 20141197, 20141198, and 20141199) were used for indexing libraries.

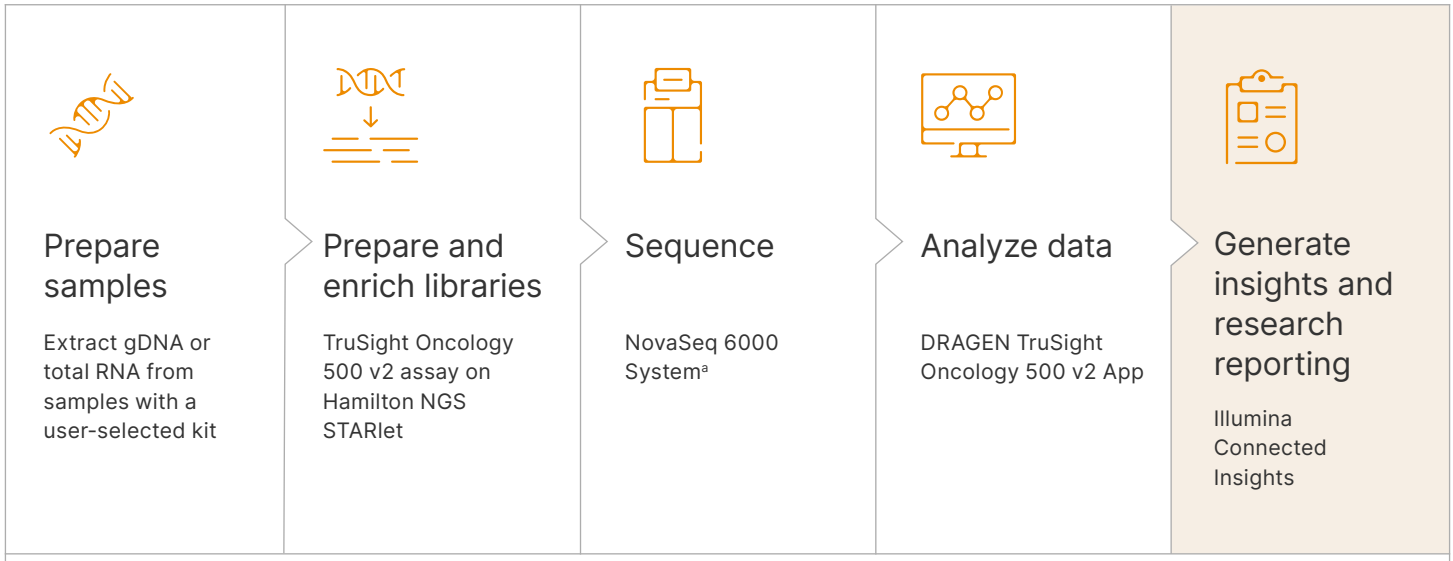

Figure 1: The TruSight Oncology 500 v2 workflow using Hamilton NGS STARlet for sample and library preparation

a. This workflow has also been tested on the NextSeq 2000 System.

TruSight Oncology RNA Control (40 ng) and ODC4 control DNA (30 ng) were used as positive template controls (PTCs) and water (for No Template Controls (NTCs). DNA samples were sheared mechanically using a Covaris ML230. Libraries were prepared using the TruSight Oncology 500 v2 method on the Hamilton NGS STARlet instrument.

Sequencing

Sequencing was performed on the NovaSeq™ 6000 System using a NovaSeq 6000 SP Reagent Kit v1.5 (200 cycles) (Illumina, Catalog no. 20040719).

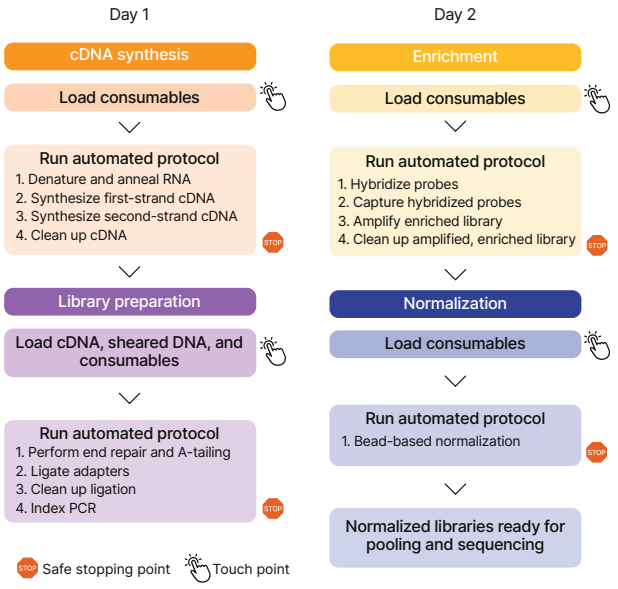

Figure 2: Automated library prep workflow with the NGS STARlet instrument
The automated workflow for preparation and enrichment of TruSight Oncology 500 v2 sequencing-ready libraries features a reduced number of user touch points (compared to manual preparation) and includes safe stopping points throughout the protocol. Enrichment steps (yellow) can be performed overnight, further reducing daily turnaround times.

Analysis

QC metrics and variant calling data were generated using the BCL Convert app (version 3.10.9) and the DRAGEN™ TruSight Oncology 500v2 app (v2.6.2.4) on BaseSpace™ Sequence Hub.

Results

Within-lab precision results

Data reproducibility was assessed for 24 RNA and 48 DNA samples using three runs conducted at the Illumina Centre in Cambridge, UK. The runs were performed by the same user on the same NGS STARlet instrument. All libraries produced in the within-lab precision studies had concentrations above the > 3 ng/µl threshold and a detectable fragment peak around the expected 300 bp library size. Key sample performance metrics for the within-lab precision study libraries are shown for RNA (Figure 3), DNA (Figure 4), and homologous repair deficient (HRD) DNA (Figure 5). Results demonstrate similar performance for manual library preparation and automated Hamilton NGS STARlet library preparation.

Between-labs reproducibility

The purpose of this study was to test the reproducibility of two different Hamilton NGS STARlet instruments across two different laboratory sites. Both runs were conducted by the same experienced user at site one (Illumina Centre, Cambridge, UK) and site two (Illumina Solutions Centre, Milan, ITA). Instruments were set up by different Hamilton Field Service Engineer and Field Application Scientist teams for both sites. TruSight Oncology DNA control was used for input.

Library and sequencing metrics for the between-labs test runs are shown for DNA (Figure 6) and DNA HRD (Figure 7) samples. All libraries passed the key sample performance metrics.

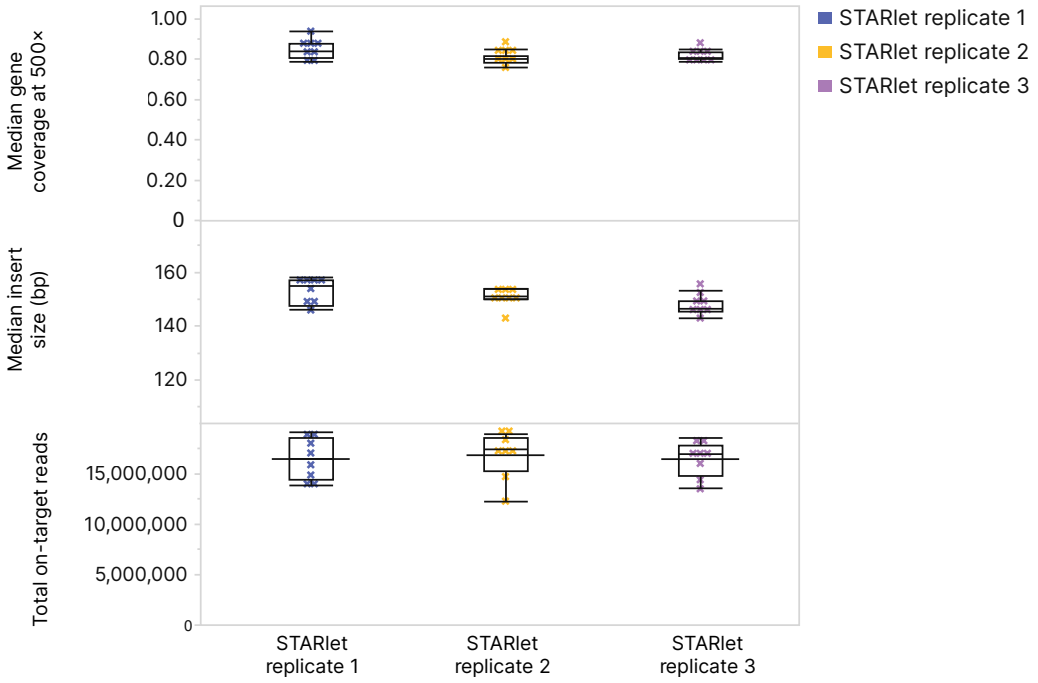

Figure 3: Within-lab RNA library precision experiments

Key sample metrics for 36 RNA libraries produced over three runs (STARlet replicate 1, 2, and 3) compared to those produced using the manual workflow, demonstrating high precision and reproducibility across the three runs, with all samples above the recommended lower specification limit guidelines.

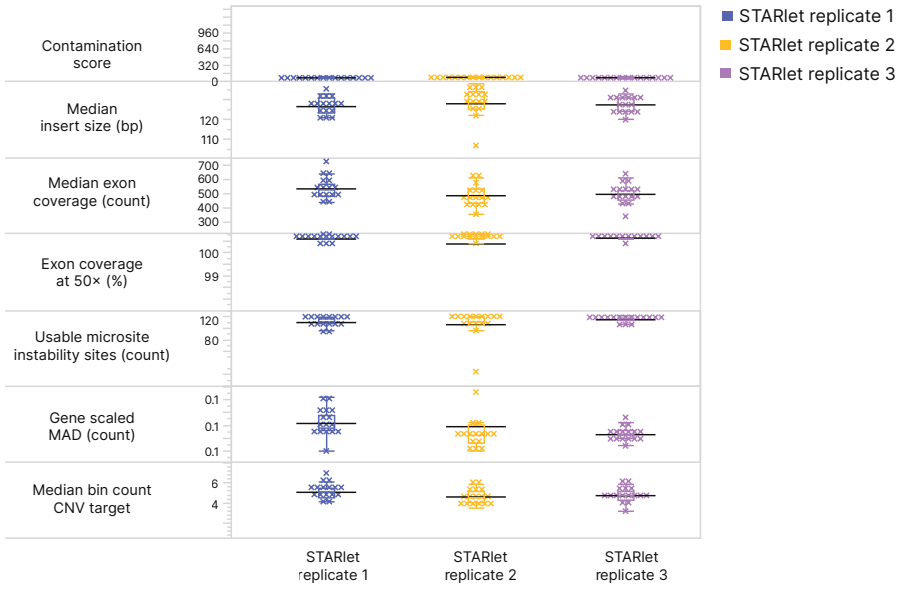

Figure 4: Within-lab DNA library precision experiments

Key sample metrics for 36 DNA libraries produced over three runs using automation (STARlet replicate 1, 2 and 3) compared to those produced using the manual workfow, demonstrating high precision and reproducibility across the three runs, with all samples above or below the recommended lower specification limit and upper specification limit guidelines.

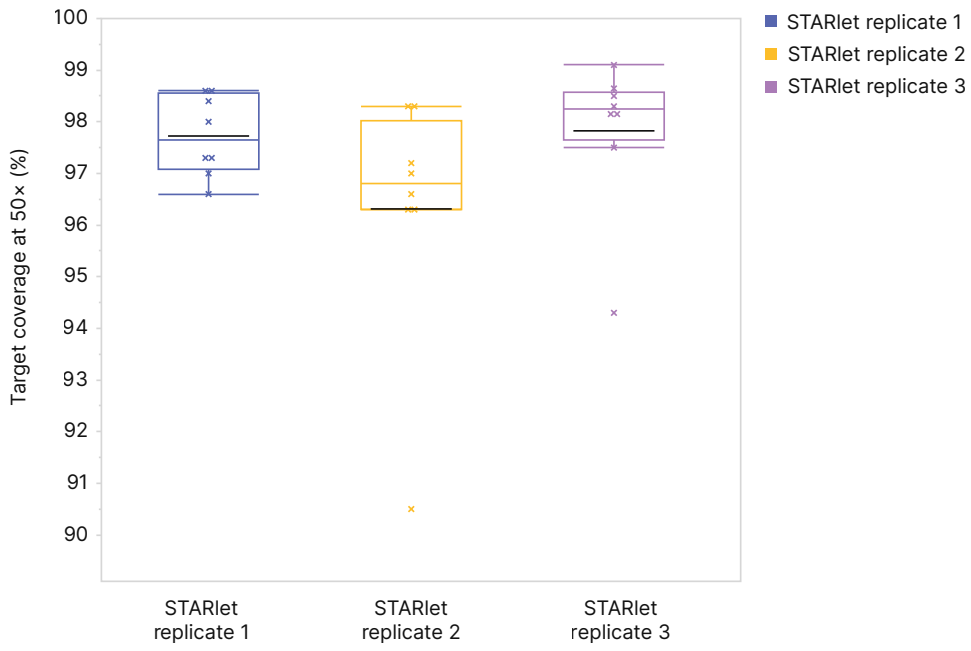

Figure 5: Within-lab DNA HRD target coverage

Libraries produced over three runs using automation (STARlet replicate 1, 2 and 3) compared to those produced using the manual workflow showed comparable homologus-repair deficiency (HRD) target coverage.

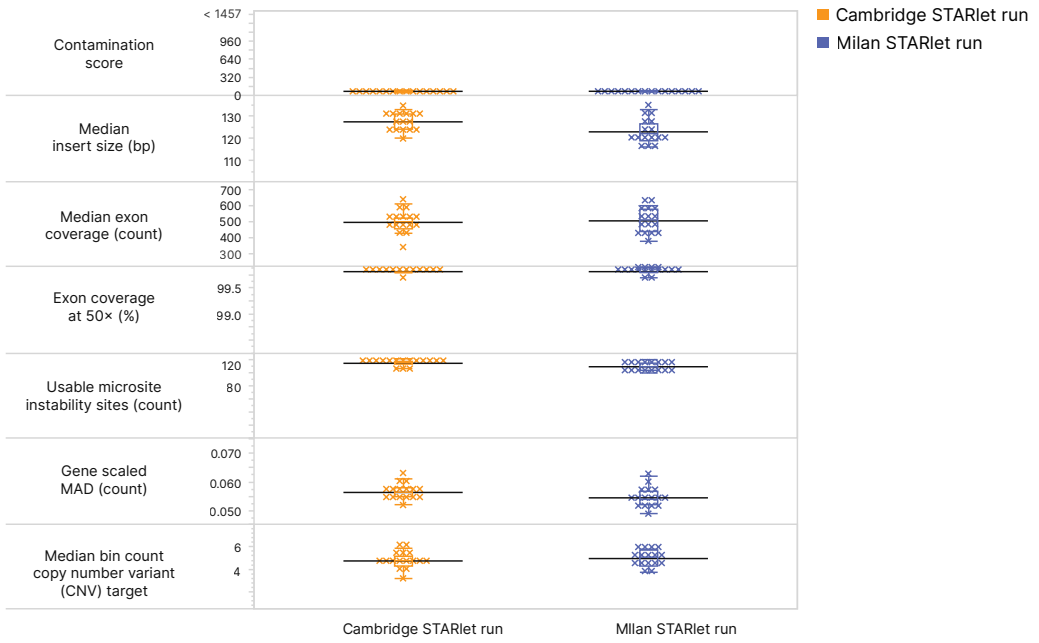

Figure 6: Between-labs DNA library performance metrics

Sequencing metrics for 24 DNA samples evaluated across two sites (Illumina, Cambridge, and Illumina, Milan) demonstrating the reproducibility of results of the method at different sites in different laboratory environments.

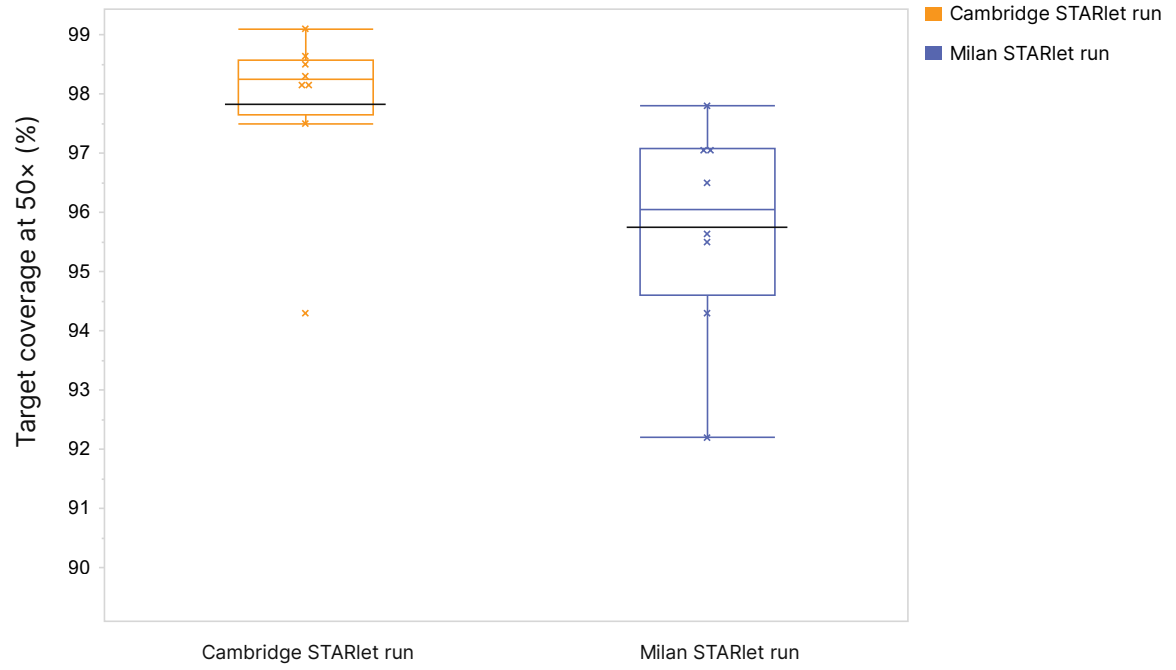

Figure 7: Between-labs DNA HRD target coverage reproducibility
Samples evaluated across two sites (Illumina, Cambridge and Illumina, Milan) demonstrating reproducible target coverage for Illumina TruSight Oncology 500 v2 libraries prepared with different Hamilton NGS STARlet instruments at different laboratory sites.

Summary

The data presented in this study demonstrate that TruSight Oncology 500 v2 libraries produced using the manual protocol or automated workflow on the Hamilton NGS STARlet liquid handler yield comparable sequencing results. Reproducible results were observed across multiple runs, sample types, instruments, and sites. Within-lab and between-labs testing confirmed this reproducibility, including using different Hamilton NGS STARlet systems. For labs performing CGP research, this technical note shows that the Hamilton NGS STARlet system is a reliable automation solution for generating high-quality Illumina TruSight Oncology 500 v2 libraries.

Learn more →

TruSight Oncology 500 v2

Illumina library prep automation

References

1. Weinstein JN, Collisson EA, Mills GB, et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet*. 2013;45(10):1113-1120. doi:10.1038/ng.2764
2. Illumina. TruSight Oncology 500 v2 data sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-oncology-500-v2-datasheet-m-gl-03460/tso500-v2-datasheet-m-gl-03460.pdf. Published 2025. Accessed May 15, 2026.

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