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TECHNICAL NOTE

Automating the TruSight™ Oncology 500 v2 assay with the Hamilton Microlab NGS STAR MOA platform

Streamlined, consistent library preparation with an Illumina Ready automation protocol

Optimized automation

Verified Illumina Ready automation protocols are ready to deploy immediately

Efficient library prep

Automated liquid-handling platforms reduce hands-on time compared to manual workflows

Concordant performance

Comparable results with automation when assessed against manual preparation

Introduction

Illumina TruSight Oncology 500 v2 is a pan-cancer next-generation sequencing (NGS) assay that enables in-house comprehensive genomic profiling (CGP) from formalin-fixed paraffin-embedded (FFPE) tumor tissue for oncology research. The assay targets 523 genes to assess DNA and RNA variant types and biomarkers, including small nucleotide variants (SNVs), insertions/deletions (indels), gene amplifications, and gene rearrangements, as well as microsatellite instability (MSI), tumor mutational burden (TMB), and genomic instability score (GIS).

Illumina has partnered with Hamilton, a leading manufacturer of liquid-handling solutions, to provide an Illumina Ready automation protocol to automate library preparation for the TruSight Oncology 500 v2 assay. These methods come fully verified and supported by Illumina, meaning customers can expect a high-quality method optimized for use without significant time investment for method adoption.

This technical note presents an Illumina Ready automation protocol for the TruSight Oncology 500 v2 assay on the Microlab NGS STAR MOA liquid-handling platform as part of a comprehensive NGS workflow (Figure 1). Results demonstrate that the automated workflow generates consistent, high-quality data with less hands-on time, compared to the manual workflow.

Methods

Samples

Automated library preparation was characterized by processing low- and high-quality FFPE tissue samples at 40 ng for RNA and 30 ng for DNA.

Library preparation

TruSight Oncology 500 v2 libraries were prepared from 10 FFPE-derived RNA samples and 10 FFPE-derived DNA samples in duplicate in three runs on each of two Hamilton Microlab NGS STAR MOA platforms. All samples were replicated in three manual preps run in duplicate (six total manual replicates) by a single operator to compare the performance of the automated and manual assays.

Sequencing

Prepared libraries were sequenced on the NovaSeq™ 6000 System (Illumina, Catalog no. 20012850) with a run configuration of 2 × 101 bp.

Data analysis

Data analysis and variant calling were performed using DRAGEN™ TruSight Oncology 500 v2.6 software. Variant interpretation can be performed using Illumina Connected Insights or other commercially available reporting platforms.

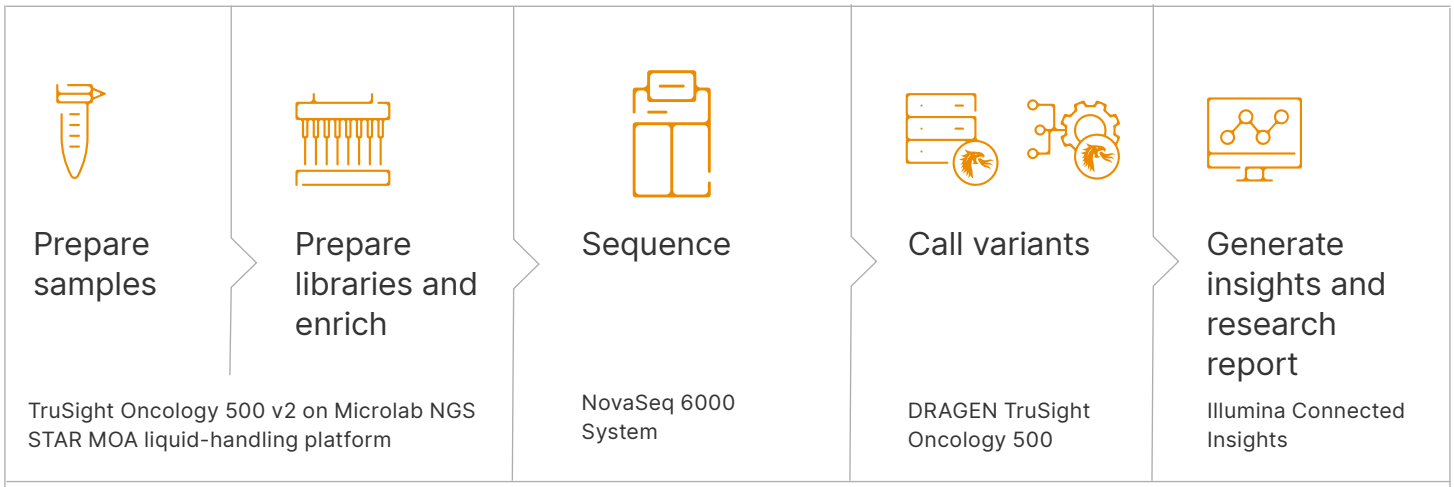

Figure 1: The TruSight Oncology 500 v2 workflow goes from sample to report in 3–4 days.

Streamlined library preparation with automation

The Illumina Ready automation protocol for TruSight Oncology 500 v2 on the Microlab NGS STAR MOA platform includes four modules—cDNA synthesis, library prep, enrichment, and normalization—that can be run with minimal touchpoints and safe stopping points throughout (Figure 2). The method can process 4–96 samples in a single batch with fully variable combinations of RNA and DNA samples, enabling an efficient workflow that can be completed in < 2 days with minimal hands-on time to prepare sequencing-ready libraries.

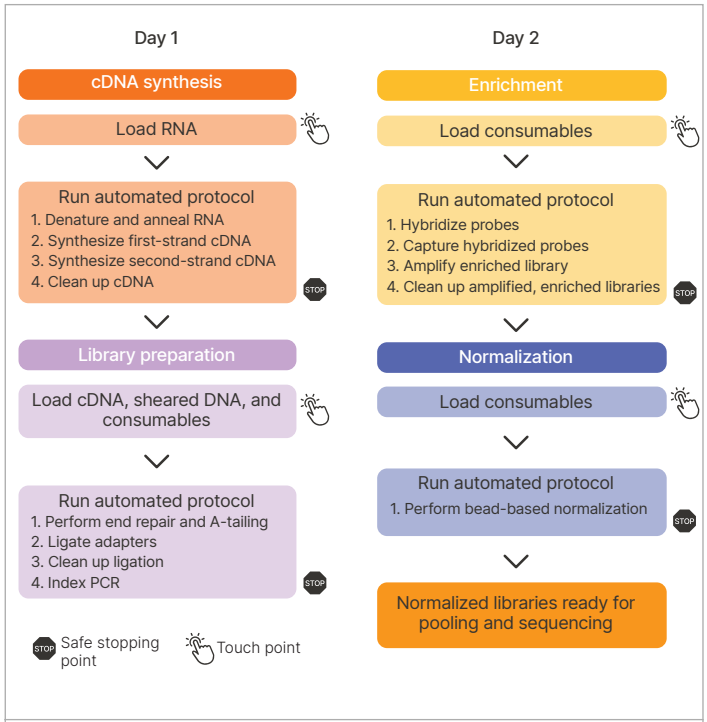

Figure 2: Automated library prep workflow with the Microlab NGS STAR MOA platform

The automated workflow for preparation and enrichment of TruSight Oncology 500 v2 sequencing-ready libraries features a reduced number of user touchpoints (compared to manual preparation) and includes safe stopping points throughout the protocol. Overnight processing is available for configurations < 48 libraries due to limitation in the number of tips available on the deck.

Results

Highly uniform and equivalent coverage

Sequencing performance of libraries prepared with the Microlab NGS STAR MOA platform were compared to performance of libraries prepared manually from the same samples on the basis of coverage. While some sample-to-sample variation was observed, exon coverage (Figure 3A) and RNA gene coverage (Figure 3B) were comparable between the automated and manual workflows for the same samples.

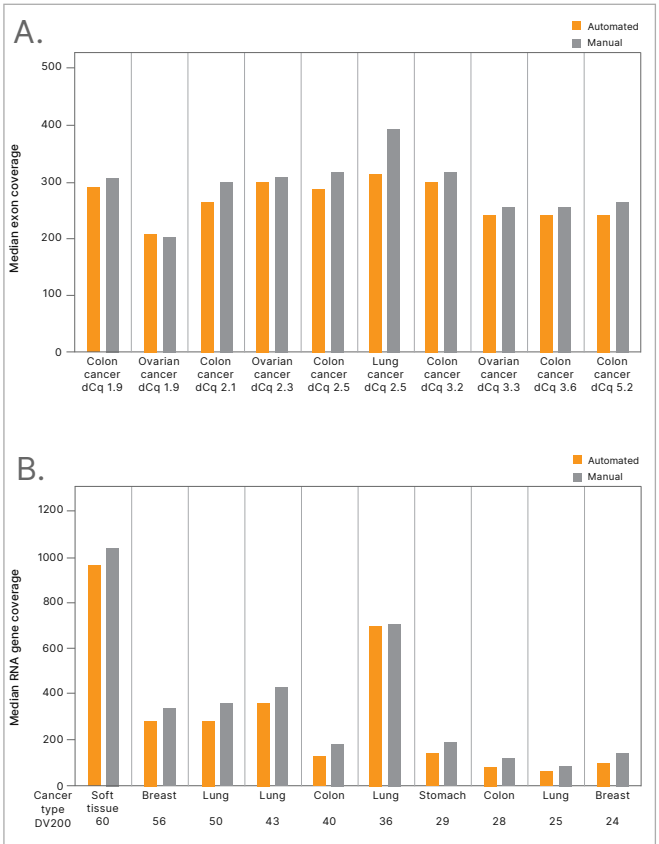

Figure 3: Equivalent coverage metrics between automated and manual workflows

Automated library preparation with the Microlab NGS STAR MOA platform resulted in comparable sequencing coverage for (A) exons and (B) RNA gene targets compared to manual library preparation for all samples evaluated.

Highly accurate and equivalent variant calling

Results show comparable performance for small variant calling, including SNVs, indels, and multinucleotide variants (MNVs) and copy number variants (CNVs) between libraries prepared with the automated and manual methods (Table 1 and Table 2) for all samples evaluated.

Equivalent detection of fusions and splice variants

Results show comparable performance for detection of gene fusions and splice variants between libraries prepared with automated and manual methods (Table 3) for all samples evaluated.

Highly concordant profiling of cancer signatures

Results show high concordance for the immuno-oncology signatures TMB (Figure 4A) and MSI (Figure 4B) between the automated and manual methods for all samples evaluated. Likewise, evaluation of homologous recombination deficiency (HRD)—a genomic signature linked to genomic instability and tumorigenesis—showed high concordance of GIS between the automated and manual methods for all samples evaluated (Figure 4C).

Table 1: Small variant detection with automation on the Microlab NGS STAR MOA platform

Tissue	ΔCq[a]	Calls/attempts and percent called by variant type[b]			
		SNV	Insertion	Deletion	MNV
Colon cancer	1.9	1097/1104 99.4%	24/24 100.0%	378/384 98.4%	N/A
Ovarian cancer	1.9	60/72 83.3%	N/A	12/12 100.0%	12/12 100.0%
Colon cancer	2.1	204/204 100.0%	N/A	N/A	36/36 100.0%
Ovarian cancer	2.3	72/72 100.0%	N/A	12/12 100.0%	N/A
Colon cancer	2.5	72/72 100.0%	N/A	N/A	N/A
Lung cancer	2.5	180/180 100.0%	12/12 100.0%	24/24 100.0%	12/12 100.0%
Colon cancer	3.2	1164/1164 100.0%	153/156 98.1%	587/588 99.8%	N/A
Ovarian cancer	3.3	66/66 100.0%	N/A	N/A	24/24 100.0%
Colon cancer	3.6	693/696 99.6%	84/84 100.0%	182/192 97.9%	12/12 100.0%
Colon cancer	5.2	564/564 100.0%	96/96 100.0%	382/384 99.5%	24/24 100.0%
Overall	N/A	99.5%	99.2%	99.2%	100.0%

a. ΔCq is a relative quantification method in qPCR that normalizes a target gene's Cq (quantification cycle) to a reference gene.
b. Reported call rates for automation-prepared libraries are all for somatic small variants with variant allele frequency (VAF) > 5%.
N/A, not applicable

Table 2: Concordant CNV detection with libraries prepared using automation on the Microlab NGS STAR MOA platform

Tissue	ΔCq	Gene	Mean fold-change	
			Manual (n = 6)	Automated (n = 12)
Ovarian cancer	1.9	*AKT2*	3.0	2.8
		CCND1	2.1	2.1
		FGF3	2.1	2.1
		FGF4	1.9	1.9
		FGF19	1.9	1.9
		MYC	2.8	3.0
Colon cancer	2.1	*BRCA2*	2.0	2.2
		FGF9	5.9	5.9
		KRAS	9.4	10.3
Ovarian cancer	2.3	*ERBB2*	2.9	3.0
		MET	2.0	2.0
Lung cancer	2.5	*FGF10*	1.8	1.9
		LAMP1	2.1	2.1

Table 3: Accurate detection of fusions and splice variants with libraries prepared using automation on the Microlab NGS STAR MOA platform

Tissue	DV200[a]	Variant	Mean supporting reads	
			Manual (n = 6)	Automated (n = 12)
Ovarian cancer	60	*ATF1:EWSR1* fusion	135	136
		EWSR1:ATF1 fusion	32	35
		EWSR1:ATF1 fusion	129	123
Breast cancer	56	*PVT1:MYC* fusion	97	73
		PVT1:MYC fusion	25	22
Lung cancer	50	*EML4:ALK* fusion	26	23
Lung cancer	43	*EML4:ALK* fusion	64	45
Colon cancer	40	*AKAP9:BRAF* fusion	71	46
		AKAP9:BRAF fusion	187	112
Lung cancer	36	*FGFR3:TACC3* fusion	1554	1594
Lung cancer	25	*KIF5B:RET* fusion	64	56
Breast cancer	24	*AR* splice variant	30	23

a. DV200 is a key quality control metric used to assess RNA integrity, representing the percentage of RNA fragments longer than 200 nucleotides.

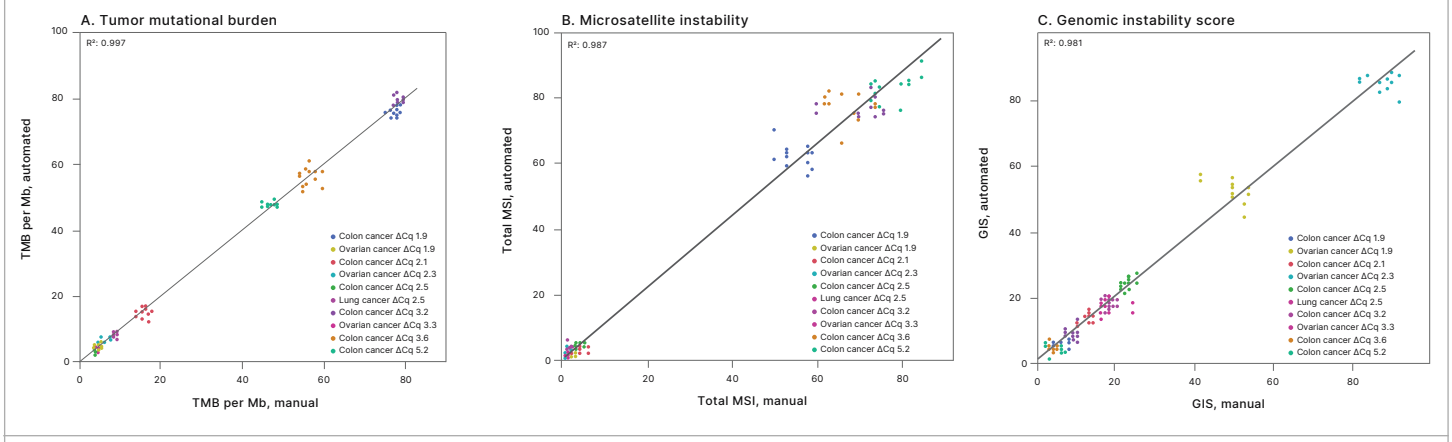

Figure 4: Concordant assessment of genomic signatures associated with cancer
Evaluation of genomic signatures associated with cancer showed high concordance between automated and manual methods for (A) TMB, (B) MSI, and (C) GIS.

Summary

Illumina has collaborated with Hamilton to provide an Illumina Ready automation protocol for TruSight Oncology 500 v2. Automating library prep reduces hands-on time to drive efficiency and provides high-quality performance. Illumina Ready automation protocols are high-quality, optimized, and validated methods that enable easy adoption. This technical note demonstrates that TruSight Oncology 500 v2 libraries prepared with automation on the Hamilton Microlab NGS STAR MOA system provide comparable performance to libraries prepared manually.

Learn more →

TruSight Oncology 500 v2

Illumina library prep automation

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