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TECHNICAL NOTE

Automating the TruSight™ Oncology 500 v2 assay with the Beckman Coulter Biomek i7 workstation

Streamlined, consistent library preparation with an Illumina Ready automation protocol

Optimized automated method

Validated Illumina Ready automation protocols are ready to deploy immediately

Efficient library preparation

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, gene rearrangements, as well as microsatellite instability (MSI), tumor mutational burden (TMB), and genomic instability score (GIS).

Illumina has partnered with Beckman Coulter Life Sciences, 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 protocols come fully validated 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 Biomek i7 workstation 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 ten FFPE-derived RNA samples and 10 FFPE-derived DNA samples in duplicate in three runs on each of two Biomek i7 hybrid workstations (Catalog no. B87579, Beckman Coulter Life Sciences). All samples were replicated in three manual preps 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 was performed using DRAGEN™ TruSight Oncology 500 v2.6 software. Variant interpretation can be performed using Illumina Connected Insights or other commercially available reporting platforms.

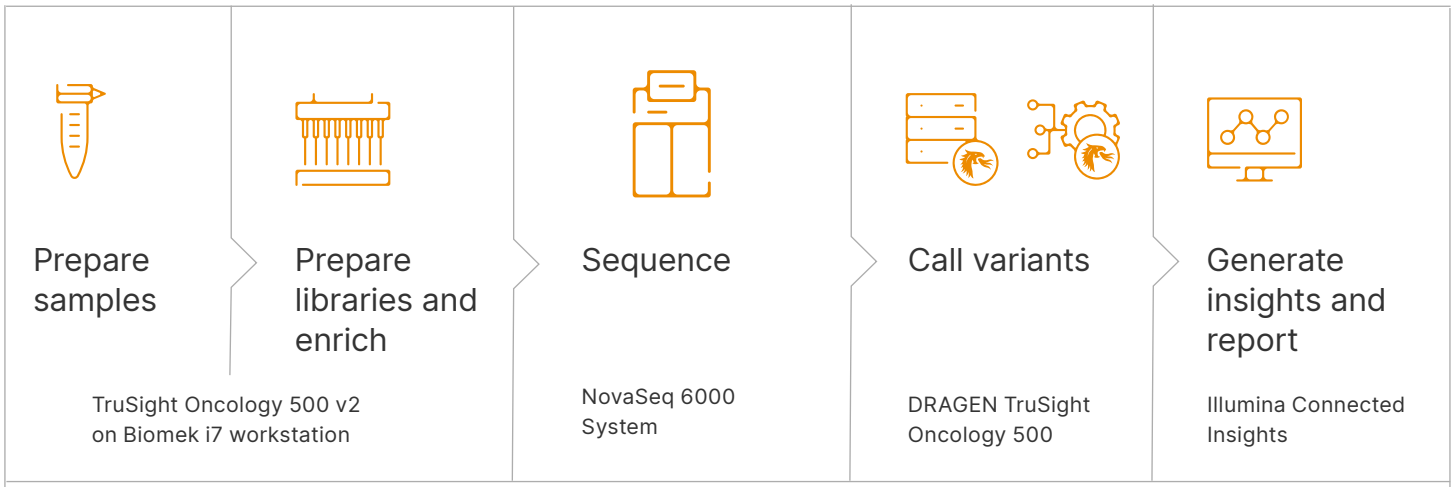

Figure 1: The TruSight Oncology 500 v2 workflow goes from sample to report in 3–4 days.

Results

Streamlined library preparation with automation

The Illumina Ready automation protocol for TruSight Oncology 500 v2 on the Biomek i7 workstation 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 Biomek i7 workstation

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.

Highly uniform and equivalent coverage

Libraries prepared with the Biomek i7 automated workflow were compared to 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.

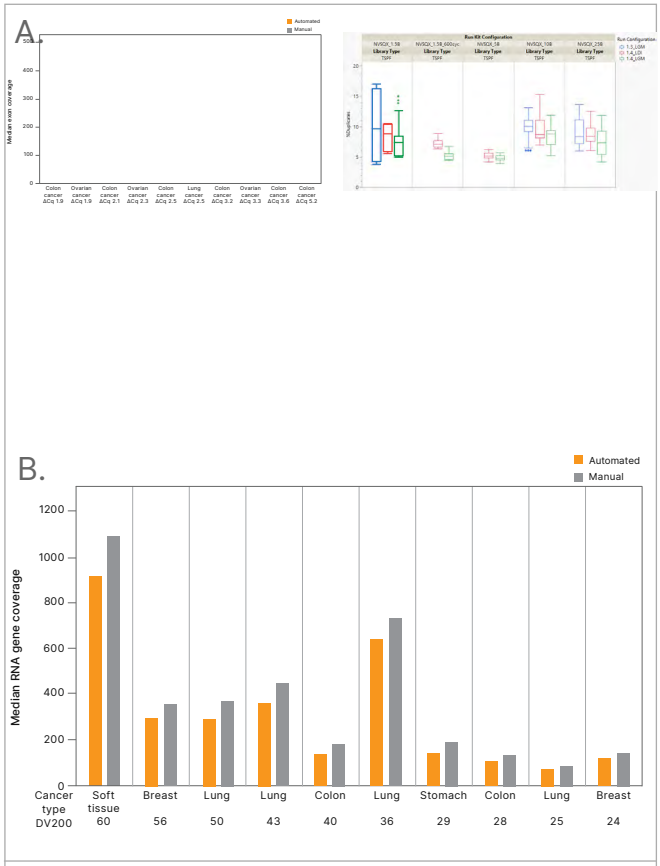

Figure 3: Equivalent coverage metrics between automated and manual workflows

Automated library preparation with the Biomek i7 workstation resulted in equivalent 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, 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 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 libraries prepared using automation on the Biomek i7 workstation

Tissue	ΔCq[a]	Automated calls/manual calls and percent called by variant type[b]			
		SNV	Insertion	Deletion	MNV
Colon cancer	1.9	1086/1104 98.4%	35/36 97.2%	411/420 97.9%	N/A
Ovarian cancer	1.9	48/48 100.0%	N/A	N/A	12/12 100.0%
Colon cancer	2.1	192/192 100.0%	N/A	N/A	36/36 100.0%
Ovarian cancer	2.3	131/132 99.2%	N/A	12/12 100.0%	N/A
Colon cancer	2.5	36/36 100.0%	N/A	N/A	N/A
Lung cancer	2.5	168/168 100.0%	12/12 100.0%	12/12 100.0%	12/12 100.0%
Colon cancer	3.2	1150/1152 99.8%	130/132 98.5%	625/636 98.3%	N/A
Ovarian cancer	3.3	84/84 100.0%	N/A	N/A	24/24 100.0%
Colon cancer	3.6	620/624 99.4%	72/72 100.0%	152/156 97.4%	12/12 100.0%
Colon cancer	5.2	612/612 100.0%	96/96 100.0%	417/420 99.3%	24/24 100.0%
Overall	N/A	99.4%	99.1%	98.4%	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%. Variant calls are reported as the number of automated calls/number of manual calls.

N/A, not applicable; SNV, single nucleotide variant; MNV, multinucleotide variant

Table 2: Concordant CNV detection with libraries prepared using automation on the Biomek i7 workstation

Tissue	ΔCq	Gene	Mean fold-change	
			Manual (n = 6)	Automated (n = 12)
Ovarian cancer	1.9	*AKT2*	3.0	2.9
		CCND1	2.1	2.1
		FGF3	2.1	2.0
		FGF4	1.9	1.8
		FGF19	1.9	1.9
		MYC	2.8	2.8
Colon cancer	2.1	*BRCA2*	2.0	2.1
		FGF9	5.9	5.6
		KRAS	9.4	9.3
Ovarian cancer	2.3	*ERBB2*	2.9	3.0
		MET	2.0	2.2
Lung cancer	2.5	*FGF10*	1.8	1.8
		LAMP1	2.1	2.0

ΔCq, a relative quantification method in qPCR that normalizes a target gene's Cq (quantification cycle) to a reference gene; CNV, copy number variant

Table 3: Accurate detection of fusions and splice variants with libraries prepared using automation on the Biomek i7 workstation

Tissue	DV200[a]	Variant	Mean supporting reads	
			Manual (n = 6)	Automated (n = 12)
Ovarian cancer	60	*ATF1:EWSR1* fusion	140	122
		EWSR1:ATF1 fusion	39	31
		EWSR1:ATF1 fusion	140	124
Breast cancer	56	*PVT1:MYC* fusion	96	71
		PVT1:MYC fusion	25	25
Lung cancer	50	*EML4:ALK* fusion	26	22
Lung cancer	43	*EML4:ALK* fusion	66	47
Colon cancer	40	*AKAP9:BRAF* fusion	69	37
		AKAP9:BRAF fusion	185	101
Lung cancer	36	*FGFR3:TACC3* fusion	1597	1272
Lung cancer	25	*KIF5B:RET* fusion	66	50
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.

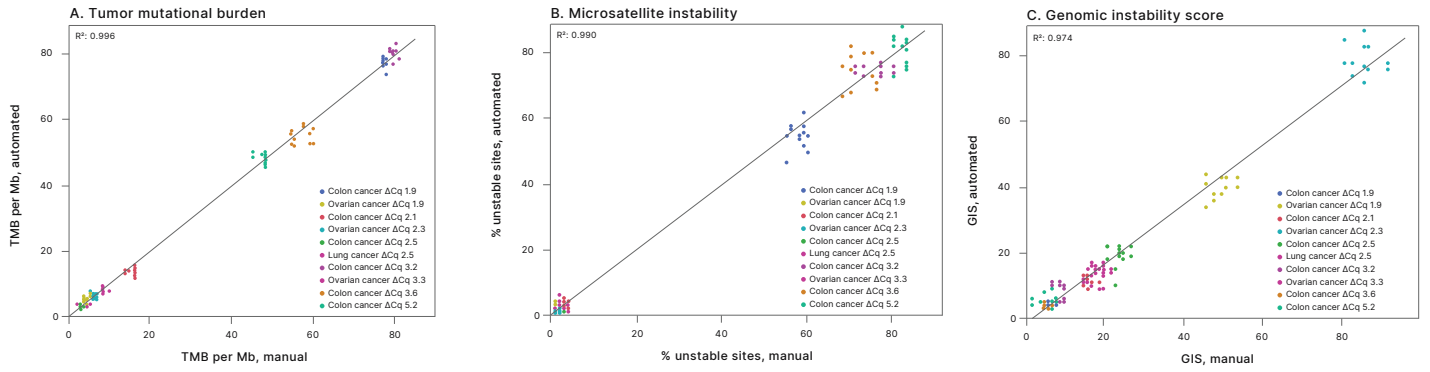

Figure 4: Concordant assessment of cancer signatures
Evaluation of cancer signatures showed high concordance between automated and manual methods for (A) tumor mutational burden (TMB), (B) microsatellite instability (MSI), and (C) genomic instability score (GIS).

Summary

Illumina has collaborated with Beckman Coulter Life Sciences to provide an automated 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 Biomek Biomek i7 workstation provide comparable performance to libraries prepared manually.

Learn more →

TruSight Oncology 500 v2

Illumina library prep automation

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-GL-04162 v1.0