BECKMAN COULTER
Life Sciences

Illumina TruSight Oncology 500 Automated on the Biomek NX^{P} Span-8 Genomics Workstation

Introduction

The TruSight Oncology 500 assay is a comprehensive next-generation sequencing (NGS) assay targeting the full coding regions of 523 genes implicated in the pathogenesis of solid tumors. Using hybrid-capture based library preparation techniques for use with formalin-fixed, paraffin-embedded (FFPE tissue) samples, TruSight Oncology 500 can analyze DNA and RNA from the same sample, detecting single nucleotide variants (SNVs), indels, amplifications, splice variants, and fusions, in a single sequencing run. TruSight Oncology 500 also allows for the determination of tumor mutational burden (TMB) and microsatellite instability (MSI) in DNA samples, which are currently being developed as biomarkers for immunotherapy. There are two library preparation kit options for the TruSight Oncology 500 assay depending on sample type: PN 20028213 (DNA kit only) or PN 20028215 (DNA and RNA kit bundle). The Biomek automated TruSight Oncology 500 workflow guarantees 32 library reactions can be prepared from the library preparation kit. If running DNA only samples, the automated Illumina TruSight Oncology 500 workflow PN 20028213 (DNA kit only) supports 3 to 32 DNA libraries in one run. If running RNA (derived from FFPE or cell lines) and DNA (derived from FFPE or cell lines) simultaneously, the automated Illumina TruSight Oncology 500 workflow PN 20028215 (DNA and RNA kit bundle) supports a combination of RNA and DNA libraries up to a total of 32 libraries. The maximum number of RNA libraries that can be processed in one run is 16. If running less than 16 RNA libraries, additional DNA libraries can be processed in one run (up to the maximum total of 32 libraries per run).

In this flyer, we demonstrate the automated performance of Illumina TruSight Oncology 500 assay on the Biomek NX^{P} Span-8 Genomics Workstation.

In comparison to the manual pipetting, automating the Illumina TruSight Oncology 500 assay on Biomek platforms provides

- Reduced hands-on time
- Reduction in potential pipetting errors
- Quick implementation with ready-to-implement methods
- Knowledgeable support

Spotlight

Biomek NX^{P} Span-8 Genomics Workstation features deliver reliability and efficiency to increase user confidence and walk-away time

- Span-8 pod with disposable tips capable of transfers ranging from 1 µL to 1000 µL
- Independent 360^{O} rotating gripper
- Orbital Shaker, peltiers, and liquid waste disposal for sample processing

Figure 1. Biomek NX^{P} Span-8 Genomics Workstation

Automated Method

The automated TruSight Oncology 500 method is constructed in a modular fashion that follows the manual assay's recommended start and stop points, allowing the operator flexibility in performing the assay and allowing the automation method to be easily deployed in pre-PCR and post-PCR laboratory spaces. Hybridization reactions and PCR reactions are performed on off-deck thermocyclers to allow for more efficient instrument utilization, but all other incubations are performed on-deck.

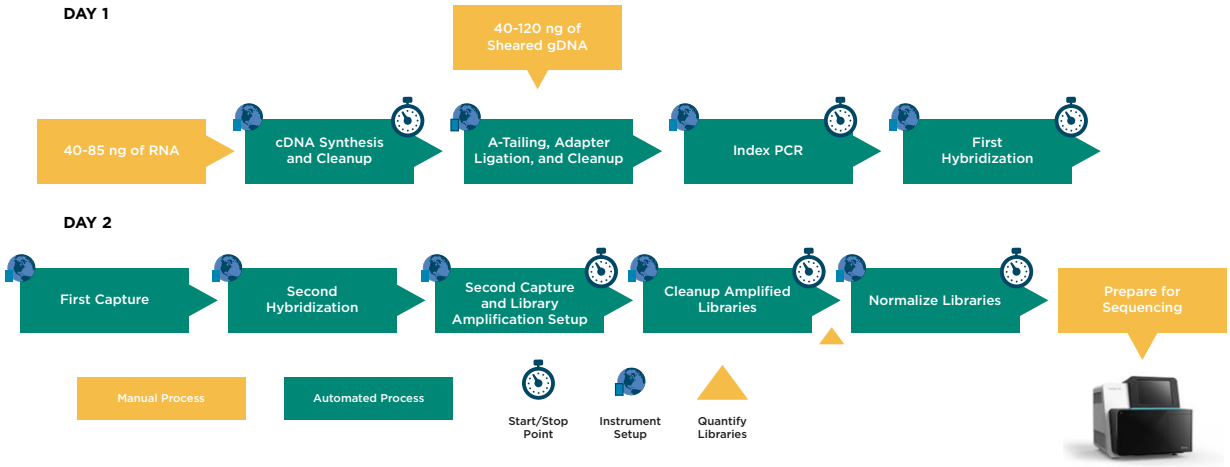

Figure 2. Illumina TruSight Oncology 500 automated method workflow

Automation provides increased efficiency, reducing the hands-on time (Tables 1 and 2).

8 DNA and 8 RNA Samples

Day 1	Time			
	Biomek Setup	**Biomek Runtime**	**Manual procedure**	**Non-Biomek Instrument Time**
Denature and Anneal RNA, First Strand Synthesis, Second Strand cDNA Synthesis, and cDNA Cleanup	15 min	2 hr, 16 min		
Covaris Shearing			15 min	2 hr
End Repair and A-Tailing, Ligate Adapters, and Clean	15 min	2 hr, 35 min		
Indexing PCR	10 min	9 min		40 min
First Hybridization	10 min	12 min		Over Night
Totals	50 min	5 hr, 12 min	15 min	>18 hr

Day 2	Time			
	Biomek Setup	Biomek Runtime	Manual procedure	Non-Biomek Instrument Time
First Capture	15 min	1 hr, 44 min		
Second Hybridization	10 min	11 min		1 hr, 30 min
Second Capture	15 min	1 hr, 8 min		
Amplify Enriched Libraries		9 min		50 min
Clean Up Amplified Libraries	15 min	37 min		
Library Quantification			20 min	
Normalize Libraries	15 min	1 hr, 12 min		
Sequencing Setup			20 min	
Totals	1 hr, 10 min	5 hr, 1 min	40 min	2 hr, 20 min

Table 1. Estimated run times for processing 8 RNA and 8 DNA samples on the automated Illumina TruSight Oncology 500 method

32 DNA Samples

Day 1	Time			
	Biomek Setup	Biomek Runtime	Manual procedure	Non-Biomek Instrument Time
Denature and Anneal RNA, First Strand Synthesis, Second Strand cDNA Synthesis, and cDNA Cleanup				
Covaris Shearing			15 min	2 hr
End Repair and A-Tailing, Ligate Adaptors, and Clean Up Ligation	15 min	2 hr, 56 min		
Indexing PCR	10 min	12 min		40 min
First Hybridization	10 min	20 min		Over Night
Totals	35 min	3 hr, 28 min	15 min	>18 hr

Day 2	Time			
	Biomek Setup	Biomek Runtime	Manual procedure	Non-Biomek Instrument Time
First Capture	15 min	2 hr, 2 min		
Second Hybridization	10 min	18 min		1 hr, 30 min
Second Capture	15 min	1 hr, 18 min		
Amplify Enriched Libraries		10 min		50 min
Clean Up Amplified Libraries	15 min	49 min		
Library Quantification			20 min	
Normalize Libraries	15 min	1 hr, 21 min		
Sequencing Setup			25 min	
Totals	1 hr, 10 min	5 hr, 58 min	45 min	2 hr, 20 min

Table 2. Estimated run times for processing 32 DNA samples on the automated Illumina TruSight Oncology 500 method

The software provides several user-friendly features such as

1. Biomek Method Launcher (BML)

BML is a secure interface for method implementation without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interact with the instrument, if needed. The application can also be run through standard Biomek software if Biomek Method Launcher is not available.

Figure 3. Biomek Method Launcher provides an easy interface to launch the method.

2. Method Options Selector (MOS)

MOS enables selection of plate processing and sample number options to maximize flexibility, adaptability and the ease of method execution

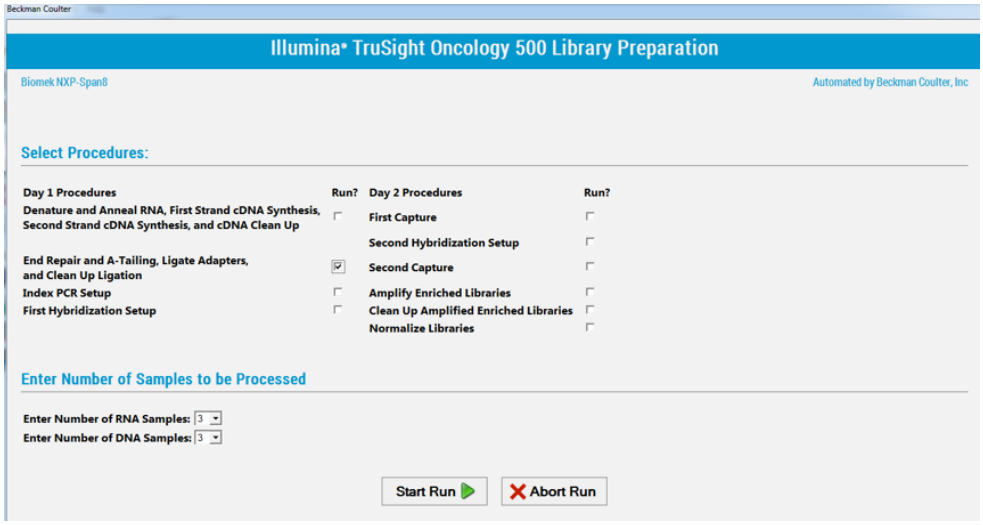

Figure 4. Biomek Method Options Selector enables us to select the desired workflow, sample number, and a variety of workflow customization options

3. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS, and provides the user specific graphical setup instructions with reagent volume calculation and step by step instructions to prepare reagents.

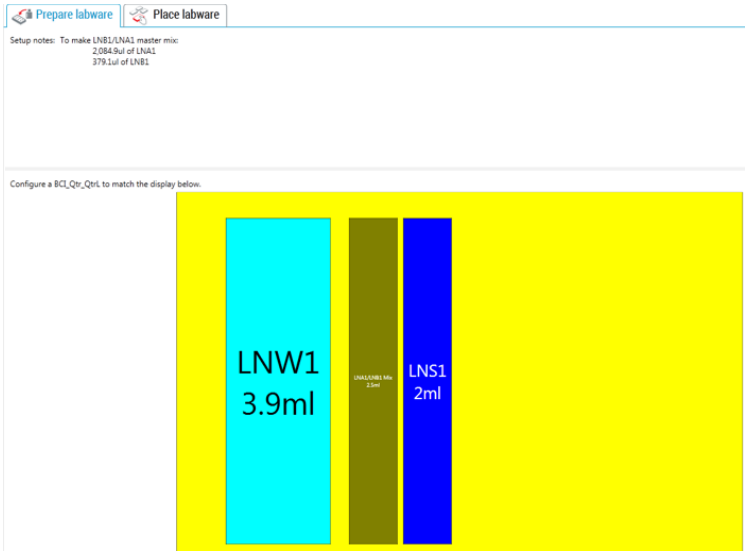

Figure 5. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup

Experiment Design

Using two Biomek NX^P Span-8 systems, a reproducibility study was performed consisting of three runs of the Illumina TruSight Oncology 500 automated method on each Biomek NX^P Span-8 along with manual controls. For each manual and automated run, 8 RNA and 24 wells of DNA samples were processed. RNA samples included four SeraCare RNA replicates (Seraseq Tumor Fusion RNA Mix v3 Part # 0710-0431) and and four FFPE RNA samples from Illumina. DNA samples included four SeraCare DNA replicates (Seraseq Tri-Lvl Mut DNA Mix v2 HC Part # 0710-0097) and 20 FFPE DNA samples from Illumina with ΔCq values ranging from 1.83 to 4.11. After library construction, sequencing and analysis was performed at Illumina. A subset of the DNA FFPE sample and cell line sample replicates are shown below, with the SeraCare DNA replicates identified as "DNA Cell Line Mix" in the following figures.

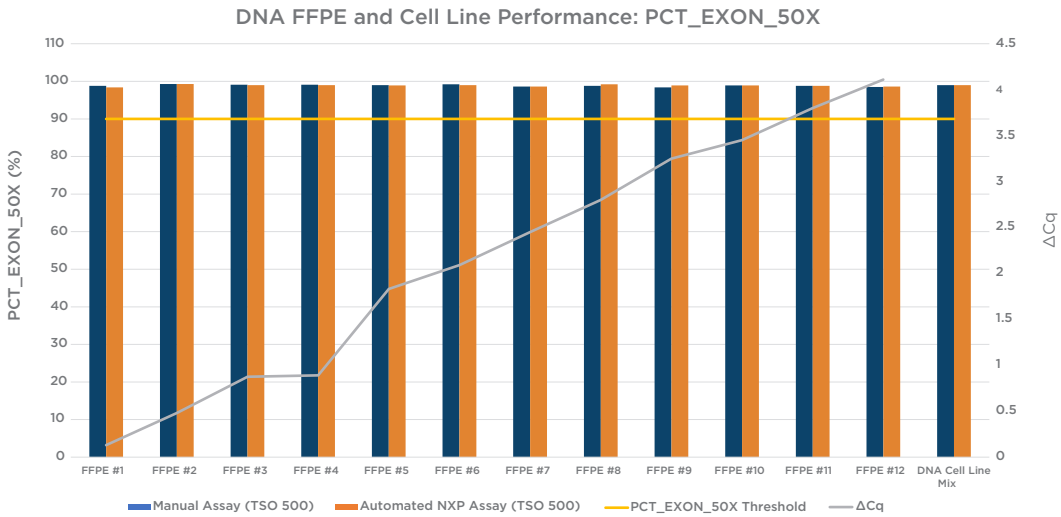

Figure 6. Percent exon coverage at 50X by sample.

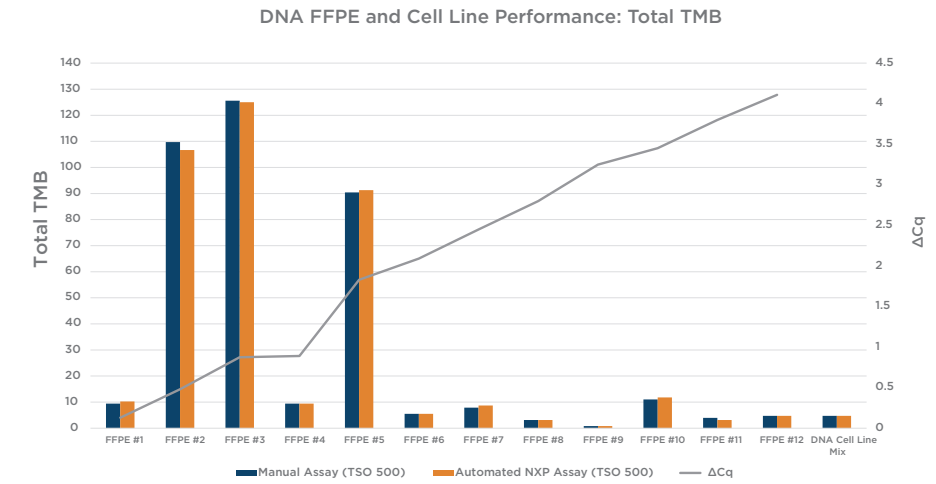

Figure 7. Total TMB (Tumor Mutation Burden) score by sample.

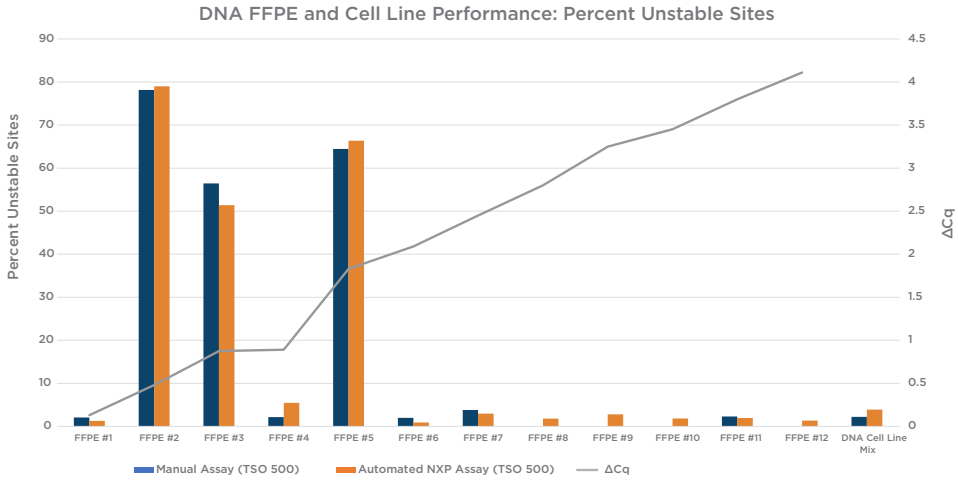

Figure 8. Percent MSI (Microsatellite Instability) sites by sample.

RNA libraries prepared on the Biomek NXP Span-8 system performed comparable to bench and passed all QC thresholds set by Illumina as shown in Table 3.

Platform	Libraries Passed AccuClear	Median Insert Size	Median_CV_GENE_500X	Total_On_Target_Reads
Manual Assay (N=24)	92%	100%	100%	92%
Automated NXP Assay (N=48)	98%	100%	98%	98%

Table 3. RNA library metrics.

Summary

A robust and reliable automation solution for NGS library prep is essential to take full advantage of Illumina's sequencing technology. Automation of the library preparation process can offer laboratories significant time savings compared to manual preparation. For example, manual user is estimated to take approximately 6 hours and 30 minutes of hands-on time to prepare 8 RNA and 8 DNA libraries, while the Biomek NXP Span-8 Genomics Workstation can prepare same number of libraries with less than 3 hours of hands-on time.

We've demonstrated automation of the Illumina TruSight Oncology 500 assay on the Biomek NX^{P} Span-8 Genomics Workstation delivers libraries that yield quality results across sample types of varying quality.

Biomek Method Launcher software package must be purchased separately.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

AAG-5610APP07.19