Automation of the Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel Kit and the Illumina TruSight™ Oncology 500 DNA/RNA Kit on the Biomek NGeniuS Next Generation Library Preparation System

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Introduction

Preparation of Next Generation Sequencing (NGS) libraries manually can be labor-intensive and time-consuming, taking anywhere from a few hours to several days depending on the type of library being created. The Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel kit utilizes proprietary amplification technology and a small panel targeting 22 genes to amplify the target regions in order to assess DNA variants. Conversely the Illumina TruSight™ Oncology 500 DNA/RNA kit uses an alternative strategy involving NGS library preparation followed by hybridization/capture using a much larger panel of 523 genes along with other markers to develop a comprehensive genomic profile of the sample. While the Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel kit offers a much shorter workflow compared to the Illumina TruSight™ Oncology 500 DNA/RNA kit, the Illumina TruSight™ Oncology 500 DNA/RNA kit provides a more comprehensive view of the genomic landscape, including assessment of fusion events, microsatellite instability (MSI) and tumor mutation burden (TMB).

In both cases, manual preparation of samples into NGS libraries involves substantial numbers of pipetting operations, depending on the assay being performed, and careful sample handling, both of which provide opportunities for errors to be introduced into the process. Utilization of an automated liquid handling system, such as the Beckman Coulter Biomek NGeniuS Next Generation Library Prep System, reduces the chances of errors while at the same time providing researchers reduced hands-on time in the library preparation process. To highlight the utility and flexibility of the Biomek NGeniuS Next Generation Library Prep System in the field of oncology research, we demonstrate the automation of both the Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel kit and the Illumina TruSight™ Oncology 500 DNA/RNA kit on the Biomek NGeniuS Next Generation Library Prep System.

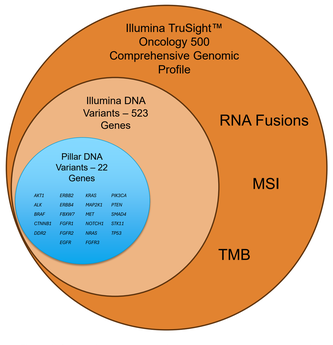

Figure 1: Schematic to represent variant types in a comprehensive genetic profile in oncology showing the overlap in the Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel kit and the Illumina TruSight™ Oncology 500 DNA/RNA kit.

Biomek NGeniuS Next Generation Library Preparation System

The Biomek NGeniuS Next Generation Library Prep System is a small-scale liquid handler purpose-built for NGS library preparation. The Biomek NGeniuS system features an integrated thermal cycler, labware transporter and reagent aliquoting to maximize walk-away time. A combination of Dynamic DeckOptix and the user-friendly head-up display guides users with real-time feedback on labware placement to ensure correct deck loading. Cloud connectivity allows operators to plan batches in the Biomek NGeniuS Portal Software, which is accessible on any Google Chrome or Microsoft Edge compatible Internet-connected computer. Batches can be sent to any Biomek NGeniuS system associated with the operator's tenant.

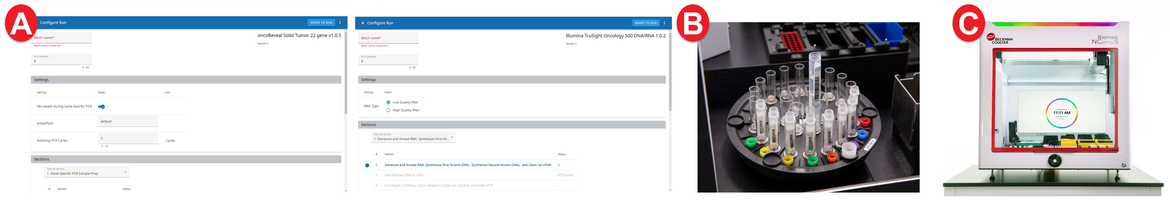

Figure 2: The Biomek NGeniuS Next Generation Library Prep System. (a) Creating a Batch in the Biomek NGeniuS Customer Portal for the Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel kit and the Illumina TruSight™ Oncology 500 DNA/RNA kit (b) Loading Biomek NGeniuS Automated Liquid Handling Workstation (c) The Biomek NGeniuS Next Generation Library Prep System Following Batch Completion.

Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel Kit

App Template Description:

The App Template for Pillar Biosciences oncoReveal™ Solid Tumor 22 gene panel prepares sample DNAs for sequencing by amplifying target regions containing mutational hot spots of 22 relevant genes using SLIMamp® (stem-loop inhibition mediated amplification) technology. The App Template allows the user to produce between four and 24 libraries in a single continuous batch run, simultaneously supporting genomic and FFPE DNA. Optionally, users may select from multiple starting and stopping points. The oncoReveal Solid Tumor 22 gene App Template supports 10-80 ng per PCR reaction for both standard genomic DNA and FFPE DNA. By the end of the first day, libraries can be sequenced on an Illumina MiSeq or NextSeq.

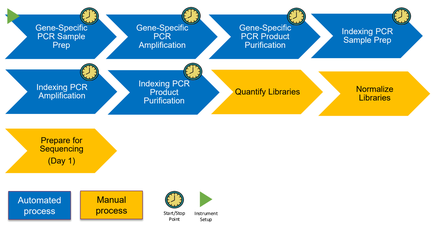

Figure 3: Automation workflow Pillar Biosciences oncoReveal™ Solid Tumor 22 gene App Template on the Biomek NGeniuS System

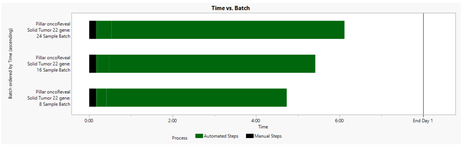

Figure 4: Estimated time of completion when running the Pillar Biosciences oncoReveal™ Solid Tumor 22 gene App Template on the Biomek NGeniuS System. Black bars represent hands-on time with the Biomek NGeniuS System.

Experiment	Batch size (samples)	Input Sample Mass (ng)	Sample Types	PCR Cycles	Instrument	Operator(s)
1	4	80	2 gDNA, 1 FFPE, 1 Nctrl	5	A	A
2	9	40	4 gDNA, 4 FFPE, 1 Nctrl	5	B	B
3	24	10	11 gDNA, 11 FFPE, 2 Nctrl	5	A	A

Table 1: Experiment configuration from the Pillar Biosciences oncoReveal™ Solid Tumor 22 gene App Template on the Biomek NGeniuS System.

Experimental Methods:

Automation of the Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel Kit was performed in conjunction with researchers at Pillar Biosciences. To test the resulting App Template, a series of runs were performed utilizing both NA18278 genomic DNA (Coriell Institute) and HD799 Quantitative Multiplex Reference Standard fcDNA (Horizon Discovery). Sequencing and analysis were performed at Pillar Biosciences utilizing an Illumina MiSeq in conjunction with the Pillar Biosciences PiVAT (Pillar Variant Analysis Toolkit) software.

Experimental Results:

A total of three test runs were performed on two Biomek NGeniuS systems at 4-, 9- and 24-sample batch sizes with 80 ng, 40 ng, and 10 ng input masses, respectively. All Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel DNA library yields were higher than the 2 nM cutoff for sequencing.

Mean coverage for all amplicons was 3330 X for fcDNA libraries and 2986 X for gDNA libraries. Effective on target rates was 94.5% and 97% for fcDNA and gDNA libraries respectively, with Q30 scores above 97%. All expected variants present in the fcDNA libraries were detected (minimum variant allele frequency was 1%).

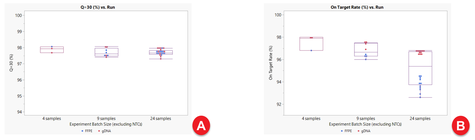

Figure 5: Sequencing metrics from libraries produced using Pillar Biosciences oncoReveal™ Solid Tumor 22 gene App Template on the Biomek NGeniuS System including (A) Q30 and (B) On Target rates.

Gene	Variant	Approximate Allele Frequency* (%)	Average Measured Allele Frequency (%), 10 ng DNA Input	Average Measured Allele Frequency (%), 40 ng DNA Input	Average Measured Allele Frequency (%), 80 ng DNA Input	Average Measured Allele Frequency (%), All Runs	Variant Detection Rate, ORST22	Average Measured Allele Frequency, TSO500 (%)	Variant Detection Rate, TSO500
BRAF	V600E	10.5	14.15	15.08	14.71	14.42 ± 1.42	16/16	12.81 ± 1.78	4/4
EGFR	ΔE746-A750	2.0	1.90	2.10	1.78	1.94 ± 0.51	16/16	1.51 ± 0.52	4/4
EGFR	L858R	3.0	4.35	4.58	4.66	4.43 ± 0.72	16/16	3.64 ± 0.86	4/4
EGFR	T790M	1.0	1.79	1.30	1.94	1.98 ± 0.38	10/16**	0.89 ± 0.25	4/4
EGFR	G719S	24.5	24.29	24.82	23.72	24.39 ± 1.21	16/16	23.23 ± 0.75	4/4
KRAS	G13D	15.0	16.48	16.13	16.37	16.38 ± 1.11	16/16	16.16 ± 2.04	4/4
KRAS	G12D	6.0	5.48	5.09	6.38	5.43 ± 0.79	16/16	5.35 ± 0.87	4/4
NRAS	Q61K	12.5	11.11	11.45	11.78	11.24 ± 1.5	16/16	10.43 ± 2.43	4/4
PIK3CA	H1047R	17.5	19.03	19.91	19.55	19.28 ± 1.32	16/16	19.43 ± 3.46	4/4
PIK3CA	E545K	9.0	8.51	8.74	8.95	8.59 ± 0.91	16/16	6.67 ± 0.75	4/4

Table 2: Observed allele frequencies and detection rate from the Pillar Biosciences oncoReveal™ Solid Tumor 22 gene App Template (blue) and Illumina TruSight™ Oncology 500 DNA/RNA App Template (green) on the Biomek NGeniuS System using HD799 fcDNA. Reported frequencies come from respective analysis software for the kits.

* The Approximate VAF% provided is for reference purposes only. The actual VAF% varies with each lot of Horizon fcDNA.
** The expected allele frequency for EGFR T790M is below the cutoff for this assay in PiVAT software. As such, a 100% detection rate is not expected. While all samples showed detection above 1%, the Average Measured Allele Frequency is calculated from the 10 variant calls passing the Allele Frequency Cutoff.

Illumina TruSight™ Oncology 500 DNA/RNA Kit

App Template Description:

The Illumina TruSight™ Oncology 500 DNA/RNA Kit App Template for the Biomek NGeniuS system allows for the creation of Illumina TruSight Oncology 500 DNA and RNA libraries compatible with Illumina sequencing platforms. The App Template allows the user to produce between four and 24 libraries in a single batch with any combination of DNA and RNA samples required. If running RNA samples, a batch requires a minimum of four touch points over the course of two and one-half working days. The user has the option of specifying if the batch contains high-quality or low-quality RNA samples and will adjust the RNA fragmentation program accordingly. Thanks to the large tip capacity of the Biomek NGeniuS System (over 1600 tips when fully loaded) and the temperature-controlled reagent storage provided by the Biomek NGeniuS system, First Hybridization through Clean Up of the Amplified Enriched Library can be run as a single operation. By running this operation overnight, libraries can be ready for sequencing by the end of the third day.

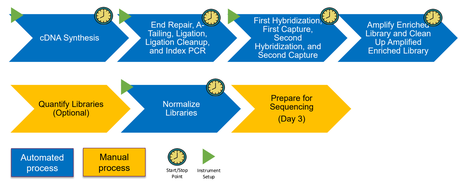

Figure 6: Automation workflow Illumina TruSight™ Oncology 500 DNA/RNA App Template on the Biomek NGeniuS System

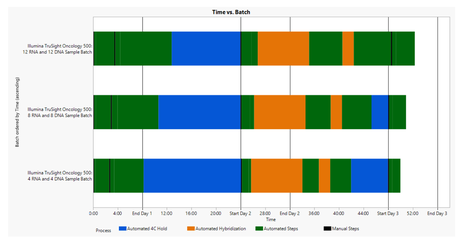

Figure 7: Estimated time of completion when running the Illumina TruSight™ Oncology 500 DNA/RNA App Template on the Biomek NGeniuS System. Black bars represent hands-on time with the Biomek NGeniuS System.

Experimental Methods:

Automation of the Illumina TruSight™ Oncology 500 DNA/RNA kit was performed by Beckman Coulter Life Sciences. A series of test runs were performed utilizing engineered control samples including SeraSeq® FFPE RNA Fusion Mix v4 (0710-0496 from LGC Diagnostics, SeraSeq® Trilevel Mutation DNA Mix v2 HC (0710-0097 from LGC Diagnostics) and Quantitative Multiplex Reference Standard fcDNA (HD798, HD799, and HD803 from Horizon Discovery). Sequencing and analysis for all replicates was performed at Beckman Coulter using an Illumina NextSeq 500 in conjunction with the DRAGEN TSO 500 Evaluation App on Illumina's BaseSpace platform and visualized using JMP and graphed with upper and lower specification limits when appropriate.

Experimental Results:

A total of three test runs were performed on two Biomek NGeniuS systems, including a 2 RNA / 2 DNA batch, an 8 RNA / 8 DNA batch, and a 12 RNA / 12 DNA batch. All Illumina TruSight™ Oncology 500 DNA/RNA library yields were higher than the 3 ng/µl cutoff for sequencing. Three sequencing runs were performed on Illumina NextSeq 500 system, with an average of 87% pass filter reads. Average Q30 scores for all three sequencing runs were 89.5%.

Analysis of RNA libraries show that all RNA libraries passed Illumina's criteria including RNA Median CV Gene 500X and RNA insert size. Across all RNA libraries, an average of 94.9% of fusion and splices present were detected.

Analysis of DNA libraries show that all DNA libraries passed Illumina's sequencing criteria. DNA Percent Exons with > 100X coverage was higher than 96% for all libraries. Usable MSI sites were 80 or greater for all DNA libraries. 99% of all variants present in the DNA libraries with variant frequency rates higher than 5% were detected.

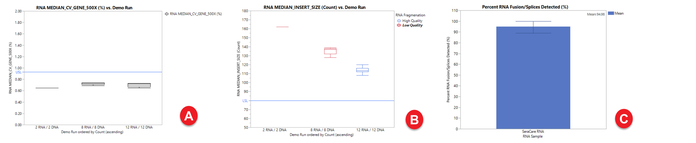

Figure 8: RNA library metrics from libraries produced using the Illumina TruSight™ Oncology 500 DNA/RNA App Template on the Biomek NGeniuS System including (A) Percent RNA Median CV Gene over 500X coverage, (B) RNA insert size, and (C) Percent RNA fusion and splice events detected.

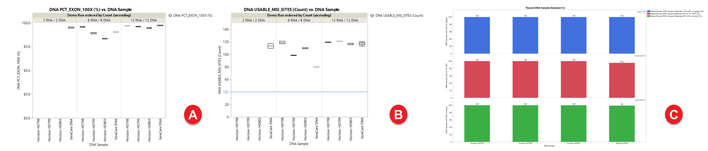

Figure 9: DNA library metrics from libraries produced using the Illumina TruSight™ Oncology 500 DNA/RNA App Template on the Biomek NGeniuS System including (A) Percent Exons over 100X coverage, (B) Usable MSI sites, and (C) Percent DNA variants detected.

Conclusion

Automation of the Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel Kit and the Illumina TruSight™ Oncology 500 DNA/RNA kit on the Biomek NGeniuS Next Generation Library Preparation System shows the flexibility of the Biomek NGeniuS System in providing cancer researchers a range of applications to facilitate their research. The Pillar Biosciences oncoReveal™ Solid Tumor 22 Gene Panel, automated on the Biomek NGeniuS system, allows the operator to begin sequencing up to 24 DNA samples on the first day for interrogating variants in 22 genes. The Illumina TruSight™ Oncology 500 DNA/RNA Kit looks at variants in 523 genes and has the ability to process RNA in addition to DNA, but the time to get samples prepared for sequencing takes longer. In both cases, the operator is freed to conduct other tasks while the Biomek NGeniuS Next Generation Library Preparation System processes the samples.

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