



Automated library preparation for the MCI Advantage Cancer Panel at Miami Cancer Institute utilizing the Beckman Coulter Biomek i5 Span-8 NGS Workstation

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Introduction

Automated library preparation allows for higher throughput and less hands-on time for researchers and clinicians, particularly for complex next generation sequencing (NGS) assays. Here we describe the automation of the MCI Advantage Cancer Panel at Miami Cancer Institute, a 170 gene panel assay based upon the Illumina TruSight® Tumor 170 Panel, on the Beckman Coulter Biomek i5 Span-8 NGS Workstation.

Miami Cancer Institute of Baptist Health South Florida is a new state-of-the-art facility which brings outpatient cancer services together under one roof to offer world-class clinical services and cutting-edge therapies. Miami Cancer Institute is Florida's only member of the Memorial Sloan Kettering Cancer Alliance. The Molecular Diagnostics Laboratory is a clinical laboratory accredited by CLIA and CAP and provides cancer genomic and routine molecular testing, by means of complex molecular analyses in oncology and bone marrow transplantation. The laboratory serves the patients at Miami Cancer Institute as well as patients throughout Baptist Health South Florida. This laboratory supports multiple disciplines and also serves as a development hub for molecular testing, allowing the lab to serve Miami Cancer Institute's patients with cutting edge technology and expertise in routine as well as specialized esoteric molecular testing.

The Illumina TruSight® Tumor 170 Panel covers 170 gene targets associated with solid tumors. The workflow includes the processing of both RNA and DNA libraries from a single sample over the course of two days (Figure 1). Following sequencing, single nucleotide variants (SNVs), indels, amplifications, gene fusions, and splice variants with a Mutant Allele Frequency as low as 5% can be identified with greater than 95% sensitivity and specificity¹. The MCI Advantage Panel combines the Illumina TruSight® Tumor 170 Panel with bioinformatic analysis performed with the TST170 apps and Variant Interpreter on the HIPAA-compliant BaseSpace Enterprise platform in addition to orthogonal analysis utilizing the Philips IntelliSpace Genomics platform.



Figure 1: Illumina TruSight® Tumor 170 Manual Workflow¹



Figure 2: Miami Cancer Institute²

Illumina TruSight® Tumor 170 Panel Automated on the Biomek i5 Span-8



The Biomek i5 Span-8 NGS Workstation from Beckman Coulter utilized for automating the Illumina TruSight® Tumor 170 Panel features 19 static positions, an orbital shaker, and two static peltiers in addition to liquid and tip waste positions. The workflow for the automated solution features modular construction to allow the same solution to be deployed on different liquid handlers for pre-PCR and post-PCR labs, or on a single liquid handler if required (Figure 3). The automated solution features Beckman's Demonstrated Method Interface, which includes Biomek Method Launcher to begin the method, the Method Option Selector to tailor the run parameters to the user's requirements, and Guided Labware Setup to indicate where labware is placed on the system (Figure 4). Finally DeckOptix helps to reduce setup errors by confirming the locations of labware on the deck using the NGS workstation's camera system (Figure 4). The automated solution can process up to 32 samples (32 RNA and 32 DNA libraries) per run utilizing two Illumina TruSight® Tumor 170 Panel kits over the course of three working days.

As can be seen from Table 1, automation of the Illumina TruSight® Tumor 170 Panel on the Biomek i5 Span-8 liquid handler results in significant time savings and increased throughput compared to manual operators.

| Process | Biomek Hands On Time (Up to 32 samples) | Manual User Hands On Time (8 samples) |
|---|---|---------------------------------------|
| cDNA Synthesis | 15 min | 70 min |
| End Repair and A-Tailing, Ligate Adapters and Cleanup | 15 min | 60 min |
| Index PCR | 10 min | 20 min |
| First Hybridization | 10 min | 10 min |
| First Capture | 15 min | 70 min |
| Second Hybridization | 10 min | 10 min |
| Second Capture | 15 min | 45 min |
| Amplify Enriched Libraries | 0 min | 10 min |
| Clean Up Amplified Libraries | 15 min | 30 min |
| Normalize Libraries | 15 min | 30 min |
| Total | 2 hours | 6 hours, 55 min |

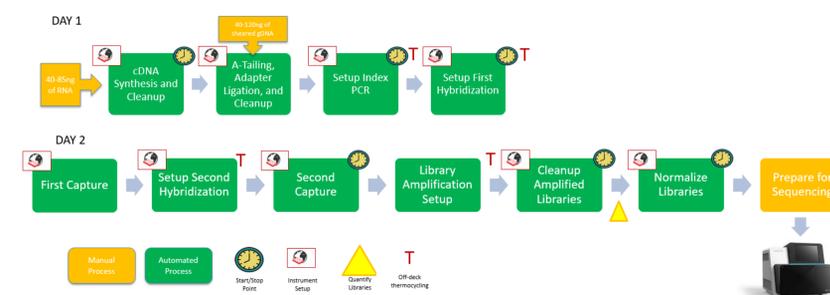


Figure 3: Automated TruSight® Tumor 170 workflow on the Biomek i5 Span-8 NGS Workstation

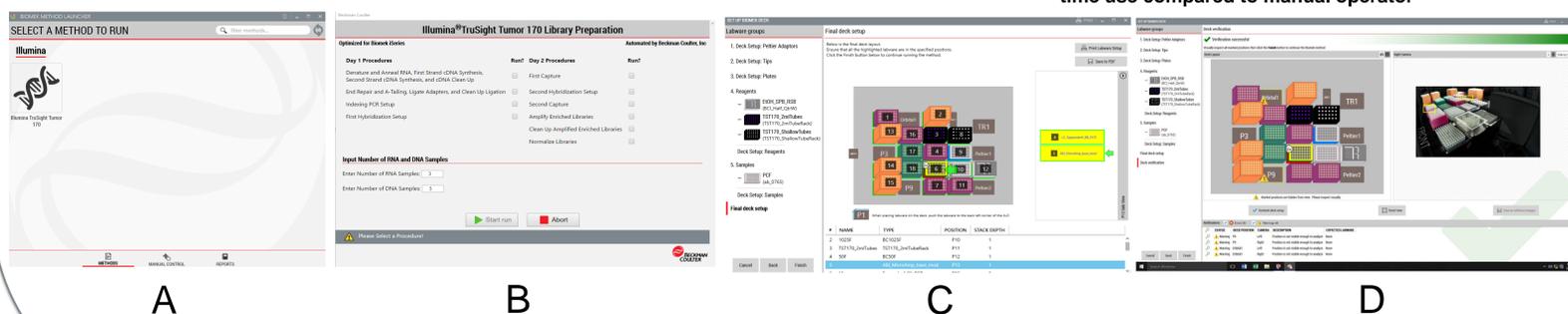


Figure 4: Demonstrated Method Interface consisting of Biomek Method Launcher dialog (A), Method Option Selector dialog (B), Guided Labware Setup dialog (C), and DeckOptix Final Check Verification (D).

Results

The automated Illumina TruSight® Tumor 170 method was installed and run at Miami Cancer Institute with a variety of samples ranging from Horizon Quantitative and Structural Reference Standards (Horizon, HD200 and HD789), Ashkenazim PGP Son Reference Standard (Horizon, GM24385), SeraSeq Fusion FFPE Reference (SeraCare, 0710-0129), and a number of in-house samples isolated with the FormaPure Total (Beckman Coulter). 108 DNA samples and 80 RNA samples were processed on the Biomek i5 Span-8 NGS Workstation over the course of 13 runs. Following sequencing, libraries were analyzed using the TruSight® Tumor 170 App on BaseSpace (basespace.illumina.com). Exon coverage at 100X or greater was very high across all DNA libraries (mean 99.78% with a standard deviation of 0.09%) as shown in Figure 5. RNA sequencing metrics (presented in Figures 6 and 7), show that for the 80 samples sequenced median insert length varied somewhat more than the DNA libraries (mean 109bp with a standard deviation of 14bp). RNA Median CV Coverage at 1000X was more consistent (mean 0.53 with a standard deviation of 0.09). RNA quality (utilizing the RNA Quality Number from the Advanced Analytical Fragment Analyzer) for the samples ranged from 10 (highest) to 1 (lowest) for the 59 samples that RQN values were available for. Purchased controls (samples 1-16) show a wide range of variability in terms of RQN as related to median insert length compared to the in-house samples (samples 17-65).

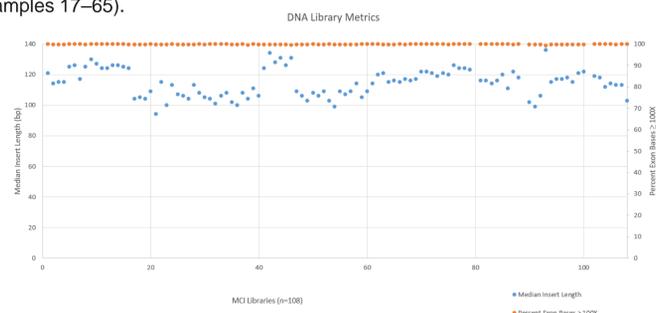


Figure 5: DNA Library Metrics

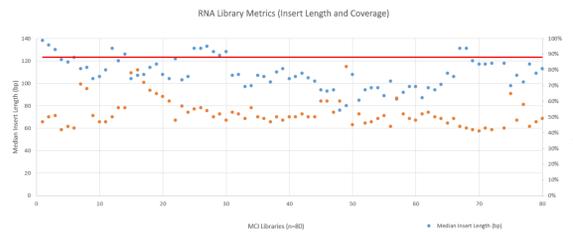


Figure 6: RNA Library Metrics (Insert Length and Coverage)

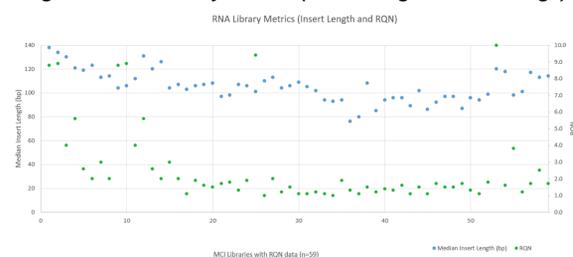


Figure 7: RNA Library Metrics (Insert Length and RQN)

83 of the samples processed using the MCI Advantage Cancer Panel were used for clinical validation. Testing using orthogonal methods such as High-Sensitivity Sanger Sequencing for EGFR, KRAS, and BRAF (LOD 10-15%) or FISH (ALK fusions) was performed at NeoGenomics. Concordance with MCI Advantage results was 98.7%. Of the two discordant results one was determined to be concordant via RT-PCR while the second discordant result (an NRAS variant) was truly discordant.

Conclusion

In conclusion, we have shown that automation of the Illumina TruSight® Tumor 170 Panel on the Biomek i5 Span-8 NGS Workstation delivers libraries that yield quality results over a variety of sample inputs while saving valuable time compared to manual operators.

References

- <https://support.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-tumor-170-data-sheet-1170-2016-017.pdf>
- Baptisthealth.net

Illumina TruSight® Tumor 170 Panel is For Research Use Only. Not for use in diagnostic procedures. <https://www.illumina.com/company/legal.html>

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