Accelerate Biomarker Discovery and Improve Results Using Automation

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ABSTRACT

Biomarkers are essential for cancer and disease diagnosis, prescribing treatment, and monitoring therapeutic effects on patients. Many genomic processes, including next-generation sequencing (NGS), are used by researchers for making Biomarker discoveries. In this poster, we describe automation solutions using Beckman Liquid Handlers for cancer biomarker discovery, saving time during NGS sample prep while increasing throughput and reducing hands-on time, as well as effective processing of challenging samples, such as formalin fixed paraffin embedded (FFPE) material.

MATERIALS AND METHODS

FFPE blocks from two breast, two lung, and liver tumor samples (all 3-5 years old) were obtained from a commercial supplier in addition to several samples of Horizon Quantitative Multiplex Reference Standard for FFPE™ (HD200). Four technical replicate 10 micron curls were obtained from each of the five FFPE blocks and transferred to Thermo Matrix tubes in addition to eight Horizon HD200 samples. FFPE DNA was then extracted using the FormaPure DNA automation method implemented on the Biomek i-Series platforms. Following FFPE DNA QC using Quant-iT PicoGreen™ (Life Technologies) and 2200 TapeStation™ (Agilent Technologies). FFPE DNA was then converted to NGS sequencing libraries using the Illumina TruSeq Exome Library Preparation Kit™ implemented on the Biomek i-Series i7 Dual Hybrid / Biomek i-Series i5 Span-8 liquid handlers. After NGS Library QC on the 2100 Bioanalyzer™ (Agilent Technologies) and ABI 7900 qPCR machine with Kapa Illumina Library Quantification Kit™ (Kapa Biosystems), six libraries were sequenced on the Illumina NextSeq™ using a 2x76bp Paired End run using High Output v2 chemistry. Sequencing data was analyzed on BaseSpace (Illumina) using the Enrichment 3.0 App (Illumina) and Variant Studio (Illumina).

RESULTS: AUTOMATED FFPE DNA EXTRACTION

FFPE Samples (Table 1) were used as template for FormaPure DNA extraction using the Biomek i7 Dual Hybrid liquid handler. FFPE DNA was then quantified using Quant-iT PicoGreen (Figure 1) and size distribution and DNA Integrity Numbers (DIN) was calculated with the Agilent TapeStation using Genomic ScreenTape (Figure 2)

Sample	Supplier	Type	Age	Technical Replicates	Curl Sizes
HD200	Horizon	Horizon Reference	N/A	8	N/A
FFPE 76	Asterand	Breast	4 year	4	10 micron
FFPE 96	Asterand	Breast	3 year	4	10 micron
FFPE 110	Asterand	Lung	4 year	4	10 micron
FFPE 121	Asterand	Lung	5 year	4	10 micron
FFPE 107	Asterand	Liver	3 year	4	10 micron

Table 1. FFPE Samples

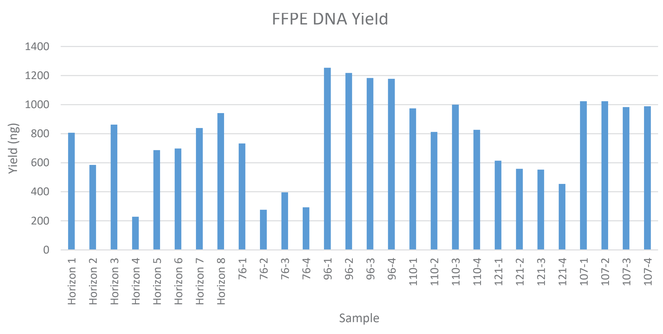


Figure 1. FFPE DNA Yields as determined using Quant-iT PicoGreen

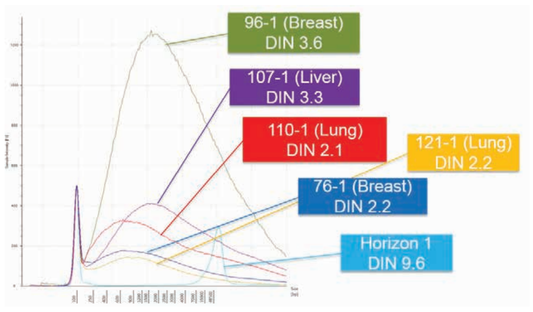
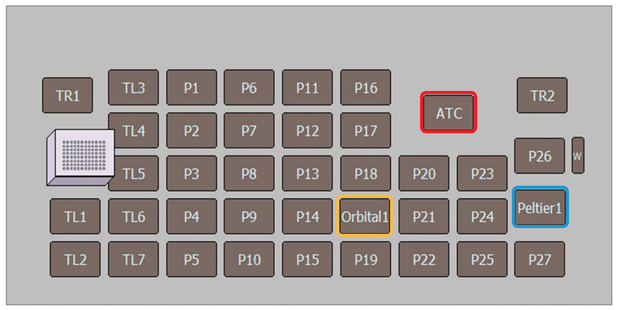


Figure 2. FFPE Size Distributions for six selected samples from the Agilent TapeStation

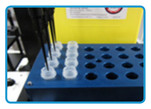
BIOMEK i-SERIES AUTOMATION



Thermo ATC

Biometra TRobot

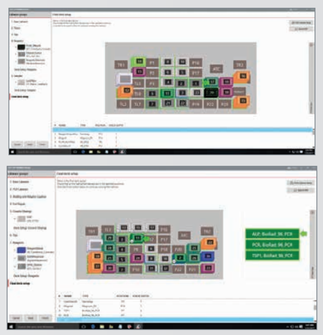
BEC Orbital Shaker



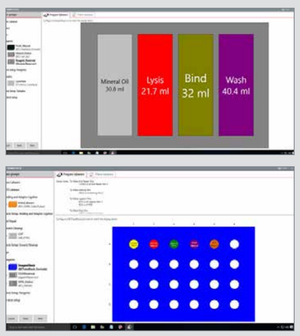
Inheco Static Peltier

The Biomek i-Series platform represents the newest automated liquid handler from Beckman Coulter. The workflow described below was performed on a Biomek i7 Dual Hybrid (left) equipped with 1200 µl multichannel pod, 1 ml Span-8 pod, and the deck layout shown above. The instrument was equipped with an orbital shaker, static peltier, and the Thermo Scientific Automated Thermocycler (ATC). The Biometra TRobot thermocycler can also be integrated as a substitution to the ATC. Biomek Method Launcher combined with the HTML Method Option Selector and Guided Labware Setup allow for ease of operation and flexibility in workflow scheduling.

Method Option Selectors



Guided Labware Setup (deck-wide)



Guided Labware Setup (individual labware)

RESULTS: AUTOMATED ILLUMINA TRUSEQ LIBRARY PREPARATION AND SEQUENCING

21 FFPE samples and three genomic DNA controls (Promega Universal Human Reference gDNA) were prepared into NGS sequencing libraries using the Illumina TruSeq Exome Library Preparation Kit using the Biomek i7 liquid handler and then subjected to hybridization / capture as four 6-plex pools. One pool was then sequenced on the Illumina NextSeq, generating 112.9M passed filter reads with 96% of those reads identified. For each library, approximately 90% of the reads mapped back to the reference genome (hg19) and 60% read enrichment was achieved (Figure 3). 80% of targeted regions were covered to the a depth of at least 20X for the FFPE block samples, while over 95% of targeted regions for the Horizon sample were covered at a depth of 20X. (Figure 4). Review of the liver FFPE sample (identified from the supplier as biliary tract carcinoma) identified mutations in seven of the top 20 genes associated with this disease (Figure 5) according to the Compendium of Somatic Mutations in Cancer (COSMIC), including TP53, KRAS, and KMT2C (Figure 6).

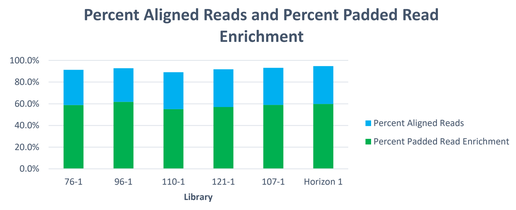


Figure 3. FFPE TruSeq Exome Sequence Alignment Results

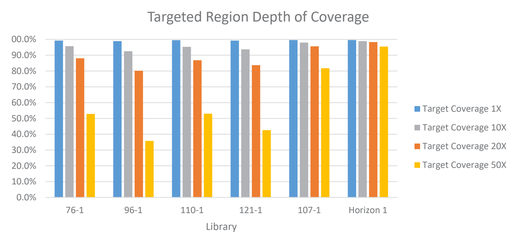


Figure 4. FFPE TruSeq Exome Depth of Targeted Region Analysis

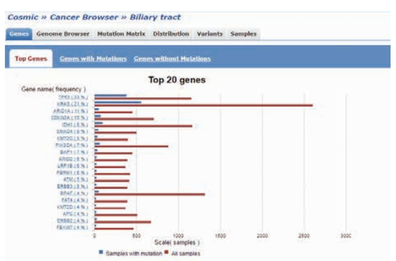


Figure 5. COSMIC Top 20 Genes Associated with Biliary Tract Carcinoma

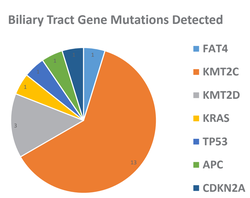


Figure 6. Gene Mutations Detected in Liver FFPE TruSeq Exome Library

CONCLUSION

In conclusion, Biomek i-Series liquid handlers offer a flexible solution for complex NGS workflows with challenging sample types.

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