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 technical replicate 10 micron curls were obtained from each of the five 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 FFPE DNA yields as determined using Quant-iT PicoGreen™ (Life Technologies) and 2200 TapeStation™ (Agilent Technologies). FFPE DNA was then sequenced using the Illumina TruSeq Exome Library Preparation Kit™ 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 at a depth of at least 20X for the FFPE block samples, while over 90% 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).

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 7 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 at a depth of at least 20X for the FFPE block samples, while over 90% 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).

CONCLUSION

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