Development of a NGS Assay for Cancer Screening Using Methylated DNA Biomarkers

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This project is to develop a novel targeted next generation sequencing (NGS) assay for early screening of Colorectal Cancer (CRC) using methylated DNA Biomarkers. CRC is the second leading cause of cancer death in the U.S., largely because early detection is hindered by low compliance rates. If detected in later stages, the 5-year survival rate is as low as 5-10%. Therefore, an urgent need exists for a non-invasive and inexpensive yet sensitive screening test. Previous studies have shown methylated DNA biomarkers such as SEPT9 and VIM as potential biomarkers for CRC. Rather than using the existing quantitative methylation specific polymerase chain reaction (qMSP) assay for detecting CRC-associated methylated DNA, this study improves the throughput, accuracy, and sensitivity of detection by using a NGS-based assay. Utilizing the NGS multiplex feature, multiple samples and genes were grouped together to increase the efficiency and decrease the cost. This assay development process consists of a Target-Specific PCR to amplify the specific regions of interest, an Adaptor PCR, followed by an Index PCR to attach barcodes to the multiplexed samples and genes, and verification of the assay through a MSP assay. The developed assay was then successfully applied to 84 urine DNA samples. The NGS data was analyzed using Genome Browse software and, when compared to the qMSP data, was proved to have a higher sensitivity, accuracy, and throughput. This is the first study to utilize targeted NGS technology to detect methylated DNA in urine samples, and the developed method can be applied for detection of other methylated DNA biomarkers. In the future, epigenetic and genetic biomarkers can potentially be combined to create one cancer panel for screening CRC and other human cancers.