Siemens Competition
Math : Science : Technology
National Finalist

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Project Title: *Simultaneous Detection of Genetic and Epigenetic DNA Modifications by Targeted Next Generation Sequencing for Cancer Screening --Assay and Data Analysis Software Development for the Detection of Hepatocellular Carcinoma*

Hepatocellular Carcinoma (HCC) is the third leading cause of cancer mortality in the United States with over 24,000 deaths annually. Due to heterogeneity of HCC, a single marker is unlikely to have sufficient sensitivity for screening. In this study we developed an assay for simultaneous detection of a panel of five genetic and epigenetic DNA modifications for HCC screening in urine by utilizing Next Generation Sequencing's (NGS) high through-put and multiplex capability. The two major limitations of NGS are the inherent error rate of 2-3% and the bottleneck of data analysis. To enhance the sensitivity of NGS in detecting mutations or methylations to 0.1 % sensitivity, we used locked or bridged nucleic acid (LNA or BNA) to suppress the wild-type templates and enrich the mutated sequence, also designed unique bisulfite specific primers in the regions that contain CpG sites for methylation detection. In order to facilitate the data analysis of millions of DNA sequencing reads, a novel software tool was developed to efficiently detect and extract mutation and methylation information of interest of the tested genes. In this study, the sensitivity improvement was validated by Sanger sequencing analysis of reconstituted standards. By using a pilot NGS data, we showed that the software was efficient and accurate in analyzing the data, thus reducing the analysis time required from 8 hours to 5 min (~100-fold reduction). The assay was then applied to 46 archived HCC patient urine DNA samples and submitted to NGS. This is the first study to successfully simultaneously detect cell-free circulating genetic and epigenetic DNA modifications using targeted NGS technology and our newly developed customizable software (fastNGSDetect) for data analysis. This method can be applied to the detection of other mutated and methylated DNA biomarkers, for other cancer detection and liquid biopsy for precision medicine to improve cancer patient care.