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Design and verification of a next generation sequencing assay to accurately detect somatic DNA variants in FFPE samples from solid tumor tissue

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Designing a next generation sequencing (NGS) DNA sequencing assay that can detect low frequency variant is a highly desired goal for therapy selection in cancer especially for the detection of actionable targets that have clinical utility. Formalin Fixed Paraffin Embedded (FFPE) tissue is the most common sample type for solid tumor histopathology. However, because the fixation process fragments DNA and damages it at varying frequencies, downstream processes can potentially misclassify modified bases and generate artifacts. We have developed a protocol that addresses both of these issues in a multiplex assay that involves deep sequencing using NGS of targets implicated in lung, gastric, colon, melanoma and ovarian cancers. A total of 200 samples were tested for 26 genes and sequencing on the MiSeq platform. The qPCR based DNA quality test, was an accurate determinant of DNA amplifiability and yielded a 99% sample success rate. A sensitivity of <5% MAF was achieved by sequencing at a minimum depth of 1,000X for all targets (average depth 20,000X). In order to differentiate true low frequency variants from fixation and other artifacts, our novel approach investigates each of the two DNA strands independently. The information was bioinformatically combined to distinguish true variants from artifacts. Testing of the FFPE samples with a 5% MAF cut off using the two strand approach reduced the potential false positive rate by ~ 40% when compared to information from only one strand of DNA. This assay, efficiently and accurately detected low frequency variants by NGS in DNA extracted from FFPE tissues.

Biography

Nitin Udar has completed his PhD from Maharaja Sayajirao University, Vadodara, India followed by a Fellowship in Molecular Diagnostics and Faculty at the University of California Los Angeles, USA. He is currently a Scientific Manager focused on Oncology within the In vitro Diagnostics (IVD) division at Illumina, San Diego, USA. He has published more than 50 papers in reputed journals as well as several book chapters.

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