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Development of quality control methods for reliable NGS measurement of methylation in cfDNA

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Abstract:
Background and Purpose: Reliable measurement of circulating free genomic DNA (cfDNA) methylation patterns promises a means for cancer early detection as well as treatment response. Use of Next Generation Sequencing (NGS) methods to measure the cfDNA methylome across a large number of potentially methylated CpG sites may enable earlier detection by overcoming stochastic sampling associated with the small specimens obtainable by a blood draw. Wide-spread adoption of these methods for clinical diagnostic applications will be facilitated through improved quality controls that increase reliability. The goal of this study is to assess the performance of the SNAQ-SEQ™ method as a means to establish a limit of detection (LOD) for measurement of methylation at each targeted CpG site. For this purpose, we used test materials that were developed by the National Institute for Standards and Technology (NIST) to mimic methylated cfDNA. Methods: NIST test materials were formulated by physically shearing cell line gDNA then subjecting it to size separation to mimic a size distribution similar to that observed in cfDNA, then mixing sheared DNA in its “native” state of methylation with sheared DNA that was in vitro methylated. Non-sheared NIST test materials also were assessed in these studies. Synthetic spike-in internal standards (IS) were combined with NIST test materials and the combined samples were bisulfite converted, then subjected to PCR-amplicon library preparation. Libraries were sequenced on an Illumina MiSeq instrument and bioinformatic analysis was conducted using the Qiagen CLC Workbench. Results and Conclusions: In initial testing of an off-the-shelf IS for a region of the SOX2 gene, average methylation (preserved C) across 14 targeted CpG sites was 16.4% (0.5%-50%) in the “native” NIST non-sheared sample. This was consistent with the 11.6% methylation across all of SOX2 measured by the NIST. In contrast, in IS corresponding to the targeted SOX2 region the average preserved C was 32.3% (0.03%-0.5%). Use of synthetic internal standards enabled determination of LOD for each individual methylated site, controlling for all known sources of technical error, including inefficient bisulfite conversion, nucleotide substitution error, and alignment error. Studies are now underway that use the NIST test materials to measure methylation at five targets known to be altered in cfDNA from lung cancer subjects, SOX2, CD81, TAC1, SOX17, and HOXA7.