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Characterizing and Developing the Clinical Grade Microbiome Assay with a Robust Bioinformatics Pipeline for Supporting Precision Medicine Driven Clinical Development

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Abstract : Purpose: It has been recognized that the microbiome plays critical roles in disease pathogenesis, including cancer, autoimmune disease, and multiple sclerosis. To develop a clinical-grade assay for exploring microbiome-derived clinical biomarkers across disease areas, a two-phase approach is implemented. 1) Identification of the optimal sample preparation reagents using pre-mixed bacteria and healthy donor stool samples coupled with proprietary Sigma-Aldrich® bioinformatics solution. 2) Exploratory analysis of patient samples for enabling precision medicine. Study Procedure: In phase 1 study, we first compared the 16S sequencing results of two ATCC® microbiome standards (MSA 2002 and MSA 2003) across five different extraction kits (Kit A, B, C, D & E). Both microbiome standards samples were extracted in triplicate across all extraction kits. Following isolation, DNA quantity was determined by Qubit assay. DNA quality was assessed to determine purity and to confirm extracted DNA is of high molecular weight. Bacterial 16S ribosomal ribonucleic acid (rRNA) amplicons were generated via amplification of the V3/V4 hypervariable region of the 16S rRNA. Sequencing was performed using a 2x300 bp paired-end configuration on the Illumina MiSeq. Fastq files were analyzed using the Sigma-Aldrich® Microbiome Platform. The Microbiome Platform is a cloud-based service that offers best-in-class 16S-seq and WGS analysis pipelines and databases. The Platform and its methods have been extensively benchmarked using microbiome standards generated internally by MilliporeSigma and other external providers. Data Summary: The DNA yield using the extraction kit D and E is below the limit of detection (100 pg/µl) of Qubit assay as both extraction kits are intended for samples with low bacterial counts. The pre-mixed bacterial pellets at high concentrations with an input of 2 x106 cells for MSA-2002 and 1 x106 cells from MSA-2003 were not compatible with the kits. Among the remaining 3 extraction kits, kit A produced the greatest yield whereas kit B provided the least yield (Kit-A/MSA-2002: 174.25 ± 34.98; Kit-A/MSA-2003: 179.89 ± 30.18; Kit-B/MSA-2002: 27.86 ± 9.35; Kit-B/MSA-2003: 23.14 ± 6.39; Kit-C/MSA-2002: 55.19 ± 10.18; Kit-C/MSA-2003: 35.80 ± 11.41 (Mean ± SD)). Also, kit A produced the greatest yield, whereas kit B provided the least yield. The PCoA 3D visualization of the Weighted Unifrac beta diversity shows that kits A and C cluster closely together while kit B appears as an outlier. The kit A sequencing samples cluster more closely together than both the other kits. The taxonomic profiles of kit B have lower recall when compared to the known mixture profiles indicating that kit B was inefficient at detecting some of the bacteria. Conclusion: Our data demonstrated that the DNA extraction method impacts DNA concentration, purity, and microbial communities detected by next-generation sequencing analysis. Further microbiome analysis performance comparison of using healthy stool samples is underway; also, colorectal cancer patients' samples will be acquired for further explore the clinical utilities. Collectively, our comprehensive qualification approach, including the evaluation of optimal DNA extraction conditions, the inclusion of positive controls, and the implementation of a robust qualified bioinformatics pipeline, assures accurate characterization of the microbiota in a complex matrix for deciphering the deep biology and enabling precision medicine.

Keywords : 16S rRNA sequencing, analytical validation, bioinformatics pipeline, metagenomics
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