

COMPARISON OF TECHNICAL APPROACHES APPLIED IN GUT MICROBIOME ANALYSES REVEALS SIGNIFICANT INCONSISTENCIES

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Gut microbiome plays an important role in affecting host physiology and in the etiology of several diseases from diabetes to autism. Microbiomics is an emerging discipline that has just recently seen its boom owing to the rapid advancements in sequencing and related techniques in molecular biology and bioinformatics. Today, a number of protocols are available for isolation, sequencing and analysis of microbial nucleic acids, and for analyzing microbial DNA- and RNA-pools. A host of information can be gained regarding a community's taxonomic composition, functional properties, transcriptomic and metabolic pathways. The process leading to the data however, is a complex series of steps that can each have different approaches, which may in turn lead to dissimilar results. Choosing the most reliable method (DNA purification kit, long- or short-read, shotgun or amplicon sequencings, ribosomal variable region choice in case of 16S rRNA V region amplicons, data analysis software etc.) is crucial at every step of the sample processing workflow, as the approach of these may differ significantly. Faced with this problem, we conducted a set of experiments comparing some of the most widely used DNA isolation protocols, sequencing and bioinformatic approaches and a bioinformatic tool, developed by our group. We have also used a microbial community mix for the validation of our results. Alpha diversity analysis was used to describe the variety and richness of the microbiota within the sample. Our results provide an optimized experimental design, a start-to-end wet-lab and bioinformatic pipeline for gut microbiome analysis.

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