Comparing effectiveness of DNA extraction kits and assessing relative abundances of microbial phyla in human fecal specimens by two next-generation sequencing platforms: key methodological steps of the "MINUTE for IBD" project from Croatia

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Introduction

The human gut contains dense and diverse microbial communities having profound influence on human health [1]. Understanding complex features of these communities includes using refined methodologies for fecal DNA sampling, isolation and sequencing and represent crucial steps in obtaining representative models of bacterial community structure and function [2]. The objective of this study was to analyze the influence that different sampling procedures, storage protocol, isolation methods and DNA sequencing platforms introduce into the final results when human feces is used.

Methods

Stool samples from 4 individuals were collected as native samples or using OMNIgene.GUT device, stored for 14 days at -20 °C (-4 °F) or at room temperature, respectively. All samples were processed with three commercially available DNA extraction kits (MO BIO Power fecal DNA isolation kit, QIAamp Fast DNA Stool Mini Kit and MP Biomedicals Fast DNA spin kit for feces). DNA yield and quality were determined by using Nanodrop and Qubit 3.0. Samples were profiled by 16S rRNA amplicon sequencing with MiSeq (illumina MiSeq, regions V3-V4) and IT (Ion Torrent PGM, regions V2-V9) platforms using manufacturer recommended reagents and protocols. QIIME and usearch61 program/algorithm were used for OTUs clustering against the GreenGenes database (version 13_8, May 2013) [3,4].

Conclusion

Overall, the effectiveness of collection, storage and DNA extraction procedures reported here are considered suitable for human feces microbiota analysis. Both native and OMNIgene.GUT samples, as well as two time points showed comparable results. MP Biomedicals extraction kit proved as most balanced across all the sample processing pipelines. However, differences between IT and MiSeq sequencing platforms could influence final microbiota composition outcomes.

References

[1] Matijašić *et al.* Int. J. Mol. Sci. 2016 Apr 19;17(4). pii: E578. **[2]** Salipante *et al.* Appl. Environ. Microbiol. 2014, 80 (24), 7583-7591. **[3]** Caporaso *et al.* Nat. Methods 2010, 7 (5), 335-336. **[4]** DeSantis *et al.* Appl. Environ. Microbiol. 2006, 72 (7), 5069-5072.

Results

Design of the study is presented in Figure 1. The average normalized DNA yield after extraction and the estimated purity of DNA varied between DNA extraction kits in the MP>QIAGEN>MO BIO order (Figure 2). A slightly higher DNA yield was obtained when using OMNIgene.GUT collection system compared to the conventional fecal sample collection, at both sampling time points (data not shown).

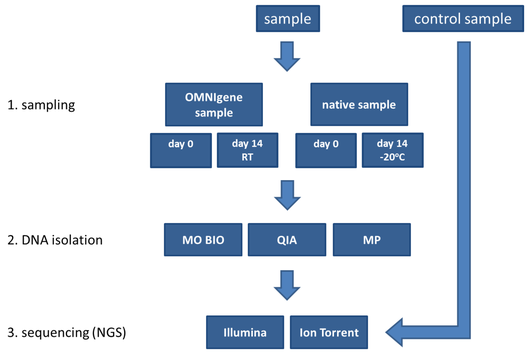

Figure 1. Schematic representation of procedures and conditions for isolation and analysis of microbial DNA from feces. The figure presents two different ways of sample collection, two different storage conditions/sampling time points, three different DNA extraction kits, as well as two different sequencing methods. RT=room temperature.

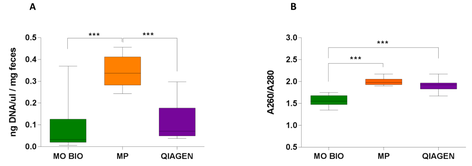

Figure 2. DNA yield (A) and quality (B) obtained using different DNA extraction kits (n=16 samples per kit). DNA yield was normalized by quantity of feces used for extraction. Bonferonni-adjusted unpaired, two-sided t-test: *** p<0.001.

Average number of OTUs per sample was 33641±3036 for MiSeq and 5843±301 for IT. Both platforms have almost all OTUs assigned at higher levels (order to phylum), but at lower taxonomic levels (species to family) higher proportion of the assigned OTUs was obtained for MiSeq sequenced samples (Figure 3). Platform-specific taxa were detected at each level accounting for the negligible proportion of bacteria identified with platforms sharing from 90% to 67% of taxa on phylum-genus levels, respectively.

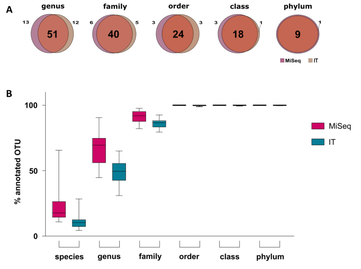

Figure 3. (A) Overlap of detected numbers of taxonomic groups on two platforms (only taxonomic groups counted minimally three times in a minimum on two samples per platform were included). (B) Percentage (%) of annotated OTUs on each taxonomic level for both Illumina MiSeq (MiSeq) and Ion Torrent PGM (IT) sequencing platforms.

Genomic DNA from Microbial Mock Community B (#HM-782D), containing equal amounts of DNA from 20 bacterial species, was used as a control sample for 16S rRNA gene sequencing on both platforms (Figure 4). Relative abundancies of several taxa deviated from the theoretically expected, the overall effect being more pronounced at IT platform. Both technologies tend to underestimate the abundance of *Actinomycetaceae* and *Propionibacteriaceae* while detecting higher proportions of *Bacteroidaceae*.

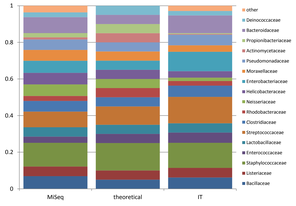

Figure 4. (left) Comparison of sequencing data from MiSeq and IT platforms with theoretical microbial mock community (#HM-782D).

The individual taxonomic content detected in samples revealed that members of the bacterial phyla *Bacteroidetes* and *Firmicutes* accounted for most of the taxon-assigned OTUs with lower abundances of *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, *Tenericutes*, *Lentisphaerae*, *Verrucomicrobia* and candidate phylum TM7. Increased *Firmicutes*/*Bacteroidetes* ratio was detected on IT when compared to MiSeq. Also, among the less abundant phyla (< 10% of the total abundance), IT identified more *Actinobacteria* and *Cyanobacteria* than MiSeq platform (Figure 5). On the family level platform related differences included the detection of more *Lachnospiraceae* and fewer *Bacteroidaceae* and *Prevotellaceae* on IT than on MiSeq platform (Figure 6A). Inter-donor diversity was detected (Figure 6B). Comparison of different collection/storage/extraction methodologies revealed that application of MP kit resulted in the least discrepancies across all the sample processing pipelines, while the use of OMNIgene.GUT collection with Qiagen extraction introduced positive and negative bias towards *Lachnospiraceae* and *Bacteroidaceae*, respectively (Figure 7). OMNIgene.GUT notably favored the isolation of *Prevotellaceae* in all the samples.

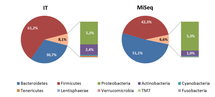

Figure 5. Summarized data of relative abundances at phylum level, averaged by platform: Ion Torrent PGM (IT), Illumina MiSeq (MiSeq). Phyla with <10% abundance are presented on each graph as a side bar.

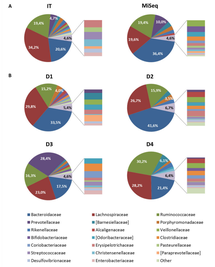

Figure 6. (left) Summarized data represented as relative abundance at family level. Data summed up by platform: Ion Torrent (IT), Illumina MiSeq (MiSeq). (A) or donor (donor 1 (D1), donor 2 (D2), donor 3 (D3), donor 4 (D4)) (B). Families with <1% abundance are presented on each graph as a side bar.

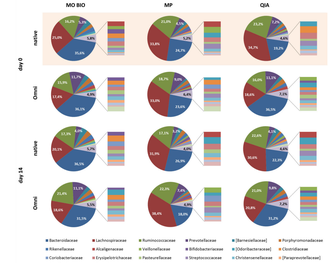

Figure 7. (up) Relative abundance of families depending on sample collection/storage and DNA extraction procedure. The graphs show DNA extraction procedures in columns (MO BIO (MO BIO), MP Biomedicals (MP), Qiagen (QIA)) while noted sampling time point (day 0, day 14) and sample collection procedure (native sample (native) and OMNIgene.GUT sample (Omni)). Families with <1% abundance are presented on each graph as a side bar.

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