Unraveling the significance of epithelial-associated bacteria in gastrointestinal diseases: Importance of choosing an optimal DNA extraction method

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Publication date:
2023

Unraveling the significance of epithelial-associated bacteria in gastrointestinal diseases: Importance of choosing an optimal DNA extraction method

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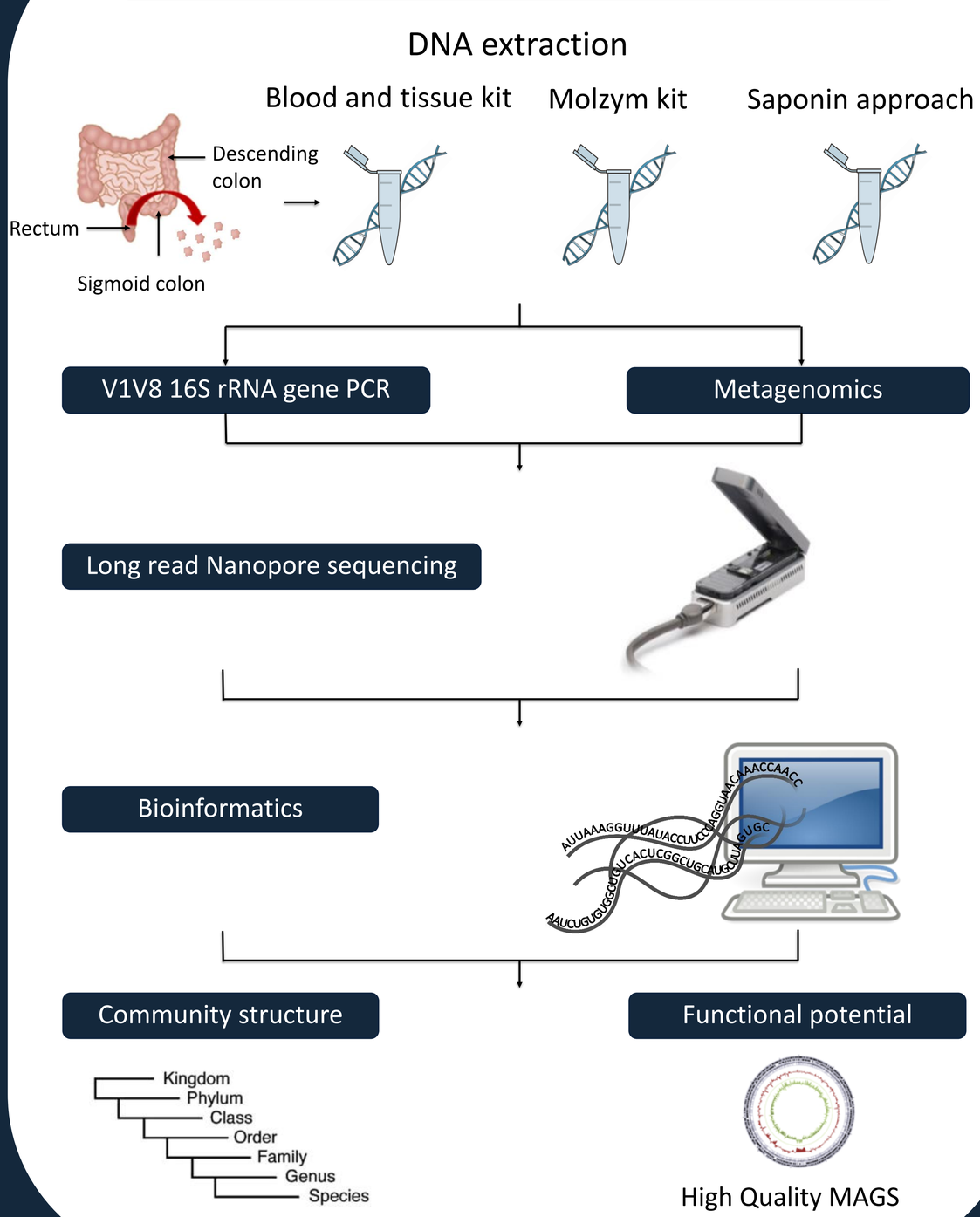
Introduction

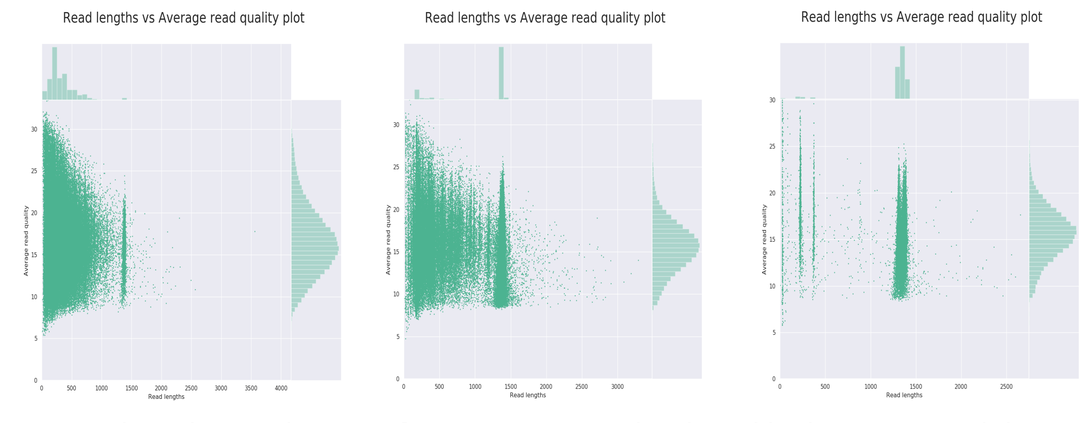
Understanding the significance of epithelial-associated bacteria in gastrointestinal diseases is essential for gaining insights into the complex host-microbiota interactions and communication that influence disease development and progression. However, sequencing approaches face limitations due to the overwhelming presence of host DNA in the samples. PCR-based approaches like 16S rRNA gene amplicon sequencing enables taxonomic profiling and has been studied extensively in connection with a wide range of diseases and while still being a valuable method, microbiome research is moving towards metagenomic sequencing. This allows for the deciphering of both the bacterial community structure at a higher taxonomic resolution as well as insight into its functional potential. The aim is to conduct a comparative study of three different DNA extraction methods for human colon biopsies: two commercially available kits (Qiagen Blood and tissue kit and Molzym ultra-deep microbiome prep) and one published optimized method (Saponin approach). The three methods were evaluated in terms of the ratio between host and bacterial DNA and their ability to retain the relative bacterial abundance at different taxonomic levels.

Methods

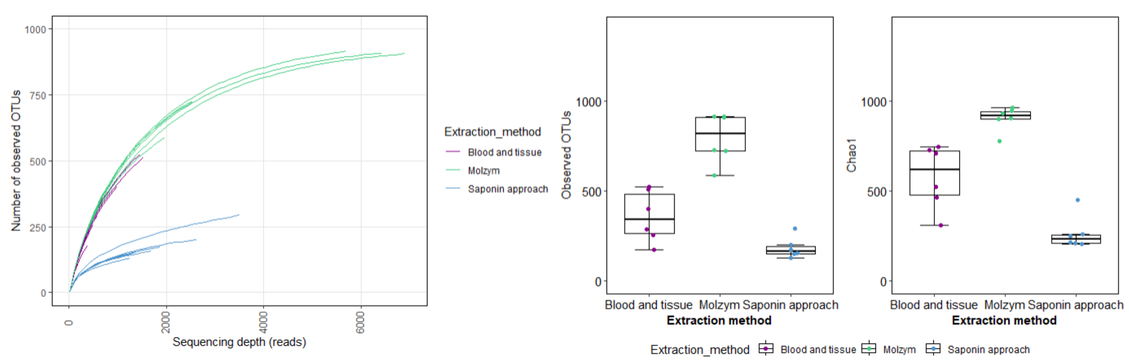


Results

- Host DNA present after 16S rRNA gene amplicon generation and sequencing

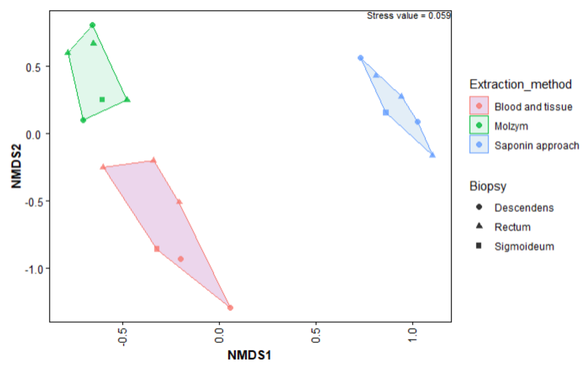


- Molzym kit reaches satisfying sequencing depth and highest captured diversity



- Molzym and Blood and tissue kit retains expected community structure

- β-diversity underlines differences between extraction method



Conclusions and Perspectives

- The choice of DNA extraction method significantly influences 16S rRNA gene amplicon and metagenomic sequencing results
- Bacterial DNA constitutes only 1-2% of the total genomic DNA in biopsies but choosing an appropriate extraction method allows for enrichment of bacterial DNA increasing the host:bacterial DNA ratio.
- The Molzym kit showed a 10-fold enrichment of bacterial DNA compared to Blood and tissue kit and is recommended for studies examining the structure and function of epithelial-associated bacteria

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