New DNA extraction workflow enables metagenomic microbiome studies on tissue samples with high host-to-bacteria cell ratio

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Background

Tissue microbiomes are an important aspect of animal biology and increasing evidence highlights their impact on host health, behavior and development. Furthermore, recent studies have demonstrated that animal tissue microbiomes undergo significant changes during disturbances in the host environment, and that the presence or absence of certain taxa correlates with factors such as temperature or anthropogenic antibiotic discharge.

Major challenges in tissue microbiome studies include:

- High host DNA background (>99%) and low bacterial biomass
- High sensitivity to contamination
- Inhomogeneous distribution of bacterial cells in the tissue matrix
- Degradation of bacterial cell envelopes during storage and transport

The presented workflow was developed based on the renowned QIAamp® DNA Microbiome Kit (QIAamp). This kit comprises a host DNA depletion step and is well established in laboratories that process samples with a high host DNA background. However, the protocol has been optimized for oral samples such as saliva, sputum and swab samples and is not recommended for tissues. Tissue samples are characterized by a complex intercellular matrix which must be dissociated to improve access to bacterial cells.

To overcome the above-mentioned issues, we have developed a specialized workflow with strong emphasis on tissue homogenization and host DNA removal. The new protocol, optimized for tissue samples (TissueP), yields high-quality bacterial DNA in quantities that allow for subsequent NGS-based analyses.

Tissue microbiome workflow

Step 1: Breakdown of tissue matrix

This is the first essential step of our new tissue microbiome protocol and is achieved via an enzymatic dissociation solution. Mechanical homogenization (e.g., using TissueRuptor® II) of each sample prior to processing is strongly recommended, to overcome inhomogeneous distribution of bacterial cells within the tissue.

Step 2: Host DNA removal

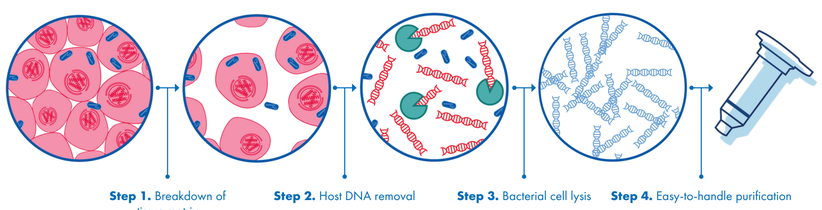
Targeted eukaryotic cell lysis is followed by enzymatic depletion of host nucleic acids, while bacterial cells remain intact. Different nucleases with DNase and RNase activities were compared, to establish an optimized, efficient host DNA depletion protocol.

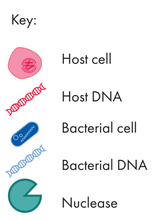
Step 3: Bacterial cell lysis

Subsequent steps include mechanical lysis of microbial cells using PowerBead Pro Tubes. During the development process, we included internal controls to ensure maximum lysis across taxa without discrimination or loss of sensitive bacterial groups.

Step 4: Easy-to-handle purification

The extracted bacterial DNA is bound to a silica-coated column to facilitate washing and purification. The binding chemistry has been optimized to remove any remaining RNA and host DNA fragments.



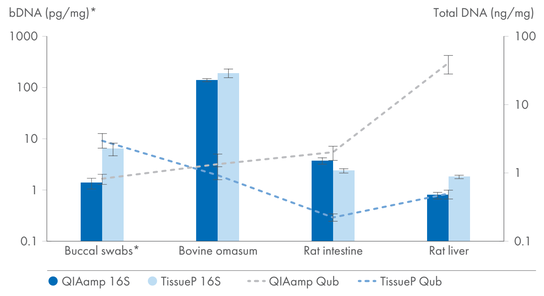


New host DNA depletion workflow for tissue samples.

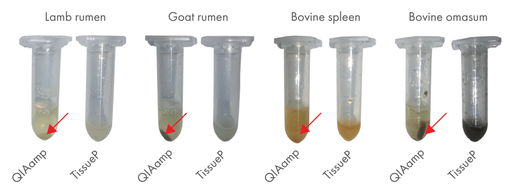
Our new workflow improves host DNA removal in soft and fibrous tissues

We have evaluated the performance of our new workflow (TissueP) in comparison to the QIAamp DNA Microbiome Kit (QIAamp), which features a host DNA removal solution optimized for sputum, saliva and swab samples. DNA yields obtained from different sample types were quantified by Qubit® measurement (total DNA, serves as a proxy for host DNA) and 16S rRNA gene qPCR (bacterial DNA).

Bacterial DNA yields obtained from buccal swab samples using TissueP or QIAamp were in the same order of magnitude, while host DNA was almost completely removed from the original sample (left figure). Total DNA yields were significantly reduced in tissue samples processed with TissueP instead of QIAamp, without compromising bacterial DNA recovery (left figure). The improved performance is attributed to the tissue dissociation step included in TissueP, which is suitable for both soft and fibrous tissues (right figure).



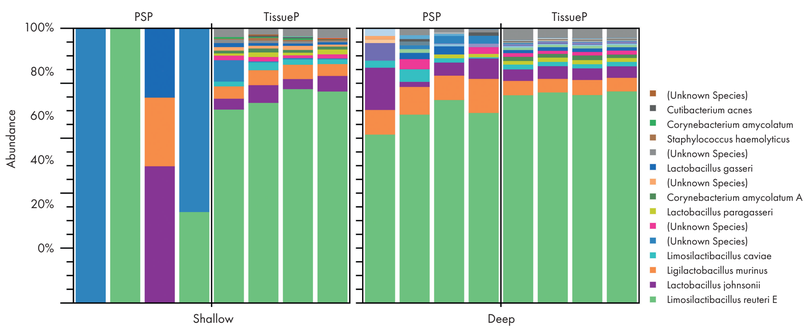
Comparison of bacterial (bDNA) and total DNA yields obtained from different sample types using the QIAamp DNA Microbiome kit (QIAamp) and the new tissue microbiome protocol (TissueP). Total DNA was quantified via Qubit (Qub) and bacterial DNA via 16S rRNA gene qPCR (16S). Values are means of technical triplicates. * For buccal swabs, unit is ng/mg for both total and bacterial DNA.



Different tissue samples after the host DNA depletion step in QIAamp and TissueP workflows. Tissues processed with the TissueP workflow were completely homogenized, while QIAamp samples still show residual tissue pieces (indicated by red arrows).

Reliable recovery of tissue microbial communities at different sequencing depths

Host DNA depletion can help keeping sequencing costs down by increasing the relative number of bacterial reads per sample. We have demonstrated that our new tissue microbiome protocol enables recovery of the main taxa from microbial communities at shallow sequencing depth (ø 26k reads/sample; see table). In comparison, much deeper sequencing (ø 2720k reads/sample) is required to adequately represent the tissue microbiome when the same sample pool was processed without host DNA depletion, for instance using the DNeasy® PowerSoil® Pro protocol. Deeper sequencing of TissueP samples captured on average 49 additional low-abundance taxa per sample, demonstrating that our new protocol preserves low-abundance microdiversity.



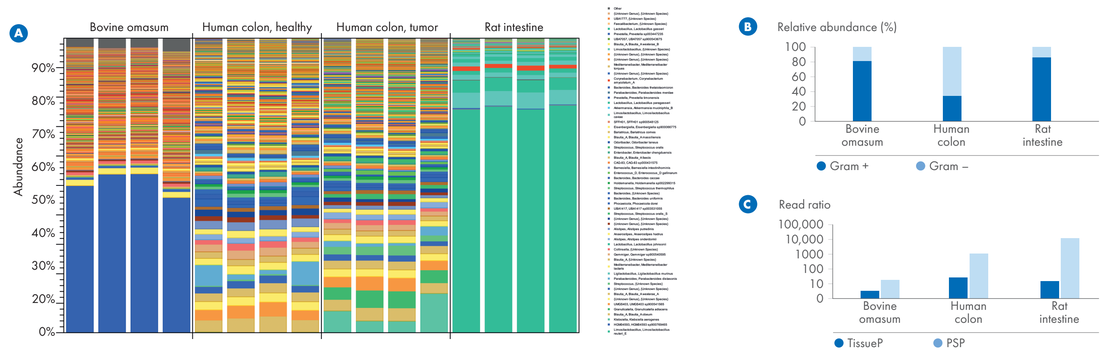
Average number of taxa found per sample in rat intestinal tissue microbiomes. Relative abundances of taxa were categorized into low (<1%), mid (≥1%) and (high ≥10%).

		Shallow QNS390	Deep QNS391
TissueP	Low	18	59
	Mid	8	8
	High	1	1
	Total	**27**	**68**
PowerSoil Pro	Low	0	5
	Mid	0	8
	High	2	2
	Total	**2**	**15**

Taxonomic composition of microbial communities in rat intestinal tissue. Tissue specimens were processed with (TissueP) and without (PowerSoil Pro [PSP]) host DNA depletion. Relative abundances were recovered from shallow (QNS390) and deep (QNS391) sequencing of the same library pool and subsequent taxonomic profiling against the UHGG database. The analysis was carried out using the CLC Genomics Workbench with CLC Microbial Genomics Module (QIAGEN, Aarhus, version 24.1.1).

Highly diverse low-abundance taxa captured by the new tissue microbiome workflow

Microbial communities in low biomass samples (e.g., animal or human tissues) are often characterized by individual taxa that are easily captured due to their high relative abundance (e.g., *Lactobacillaceae*, **A**). We have demonstrated that our workflow enables, in addition, recovery of highly diverse low-abundance Gram-positive and -negative bacteria (**A** and **B**). Thereby facilitating discrimination between largely similar samples (e.g., human colon adenocarcinoma/healthy adjacent tissue, **A**). This high resolution is achieved by increasing the relative number of bacterial reads via host DNA removal (**C**).



Analysis of whole genome sequencing data from different tissue samples processed using the TissueP workflow. A Taxonomic profiles (species-level) assigned by matches with the UHGG database (carried out with CLC Genomics Workbench and CLC Microbial Genomics Module). **B** Average representation of Gram-positive and -negative bacterial taxa in the sequenced samples. **C** Host-to-bacterial read ratio in samples processed using TissueP and PowerSoil Pro (PSP). Higher taxonomic resolution is achieved by improving the relative number of bacterial reads in TissueP samples. Depicted values are means of quadruplicates.

Summary

Our new tissue microbiome workflow is applicable for tissue and buccal swab samples. It is characterized by improved host DNA removal, providing several advantages:

- **Suitable for diverse sample types:** Homogenization and host DNA removal show excellent results for both soft and fibrous tissue, as well as swab samples
- **Increased number of relative bacterial reads in downstream NGS applications:** Major taxa can be captured even at relatively shallow sequencing depth
- **Unbiased:** No discrimination of taxa based on cell-wall structure. Archaea can also be recovered (data not shown)
- **Contamination free:** All reagents supplied are tested frequently for microbial DNA background

Have questions? Want to become a field tester? Contact Janina Leinberger

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Sample to Insight