The human microbiome in 2015

The latest tools for isolation and analysis of microbial DNA

Introduction to the human microbiome

The human body is populated by 100 trillion bacteria, archaea, fungi, protists, and viruses, which play a fundamental role in our well-being. The term "microbiome" refers to the microbes in those communities and their genes, as genetic analysis is the most straightforward method of identifying microbiome members. Deviations from healthy microbial compositions have been linked with many human diseases, including inflammatory bowel disease, obesity, cancer, asthma, diabetes, and allergies. Hundreds of academic institutions, as well as biotech and pharmaceutical companies, are investing in programs around gastrointestinal (GI) tract, skin, oral, and urogenital microbiomes.

Although our understanding of the microbiome and its interaction with the host is still in the nascent stages, it is becoming increasingly clear that we need to treat those interactions as a sophisticated system, much like the circulatory and immune systems, that exists in harmony with homeostasis, playing multiple roles within a human body. Thus, fundamental research is highly important: we need to gain a better understanding of microbiome composition, functions, its interaction with the human body, and explore strategies to modulate the system.

Next-generation tools, such as improved kits for isolation of microbial DNA (from stool, body fluids, and various swabs), sequencing, and data analysis, are urgently required. As fundamental research progresses, microbiome therapeutics will likely become a reality within the next few years; the microbial diagnostics market is rapidly expanding already.

Microbiome research workflow

The typical human microbiome research workflow is listed below, along with our latest kits, reagents, and instrument options most useful for these kind of studies:

1. **Sample collection.** A number of sample collection devices for the analysis of the microbiome in stool, urogenital, and oral samples are offered under the Thermo Scientific™ Sterilin™ brand. Invitrogen™ Ambion™ conical tubes (50 mL and 15 mL) may also be used for collection of human, as well as environmental microbiome, samples. The tubes are RNase- and DNase-free, sterile, nonpyrogenic, and can be autoclaved to 121°C.

2. **Sample storage.** Freezing (typically –80°C) is by far the most popular approach used by scientists for sample preservation. An alternative approach is using stabilizing solutions, which allow sample storage at ambient temperature. Invitrogen™ Ambion™ RNA*Later*™ Stabilization Solution is one of the most widely used of these reagents. It was originally designed for preservation of RNA in fresh tissue, but has been found to be useful for stabilizing many microbiome samples of human and environmental origin, as well as for DNA stabilization.

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3. **Purification of aggregate microbial DNA.** This is the key step in the microbiome research workflow, and will be discussed in more detail below. Improved kits for DNA purification are of paramount importance to detailed studies of microbial communities populating the human body.

4. **Initial DNA analysis.** The quantity and purity (260/280 nm and 260/230 nm absorbance ratios) of recovered DNA can be analyzed using the Thermo Scientific™ NanoDrop™ spectrophotometer, which requires only a 1 µL sample for spectral analysis. An alternative is to use an Invitrogen™ Qubit™ fluorometer and the highly sensitive Invitrogen™ Qubit™ DNA quantitation assay to accurately measure DNA in a 0.5–1 µL sample. The concentration of the DNA in the sample is reported by a fluorescent dye that emits a signal only when bound to the target, which minimizes the effects of contaminants—including degraded DNA or RNA or non–nucleic acid material—on the result.

   Agarose gel electrophoresis provides additional information regarding the size distribution and integrity of recovered DNA. Invitrogen™ ReadyPouch™ Agarose Gels are packaged in a ready-to-microwave pouch that contains the right amount of agarose and buffer for a 100 mL gel. They come with an easy-to-use stain dropper. All that needs to be done is vent the pouch, heat in a microwave oven, add stain, and pour.

5. **qPCR or sequencing and subsequent data analysis.** For the downstream analysis, qPCR (with Applied Biosystems™ TaqMan™ Assays) and microarrays are routinely used for detection and quantitation of predicted microbial species in the samples, whereas next-generation sequencing is used for discovery. Among the most popular next-generation sequencing technologies are Ion PGM™ and Ion Proton™ benchtop sequencers, which have reads that are sufficiently long to permit data generation from highly informative sections of the 16S rRNA gene.

To summarize, we offer a complete collection of tools and instruments to address the needs of the microbiome research community, and to enable straightforward and robust DNA isolation and analysis.

Challenges with isolation of microbial DNA

The major challenge with microbiome samples is not the ability to extract nucleic acids per se, but the isolation of a DNA sample that accurately reflects the representation of the diverse microbe populations in the community sampled. Microbes substantially differ in the compositions of their cell walls, which are the major impediments to lysis. For instance, gram-negative bacteria can often be efficiently lysed with heat alone, whereas gram-positive bacteria, with their thicker and more complex cell walls, often require an additional mechanical, enzymatic, or chemical lysis. The research community agrees that protocols that include upfront mechanical disruption of bacteria by bead beating are the best option, as this ensures the breakage of the most durable microbe known, mycobacteria.

In addition to the efficiency of lysis, the purity of the DNA can affect the downstream analysis, as some methods carry over more inhibitors of the enzymes employed in the assays being used (e.g., polymerases, ligases, phosphatases). Such inhibitors include bile, bilirubin, digested food, and humic acids, and they vary substantially in terms of size and charge, making it difficult to eliminate them without compromising DNA yield and maintaining a rapid workflow. Among the most challenging samples are stool and soil, which both contain high concentrations of diverse inhibitory substances.

A novel kit for purification of high-quality microbial DNA

To address the research community's need for a robust microbiome DNA purification method, we developed the Invitrogen™ PureLink™ Microbiome DNA Purification Kit. The PureLink Microbiome DNA Purification Kit enables fast purification of high-quality microbial and host DNA from a wide variety of sample types, including particularly challenging samples such as stool. The kit uses proven Invitrogen™ PureLink™ spin column technology for excellent yields of purified DNA ready for downstream PCR, sequencing, or other applications. The highly efficient triple lysis approach, fast removal of inhibitors, and versatility make this a superior kit for microbiome research, as well as for research programs aimed at rapid detection of pathogenic bacteria in various samples.

Features of the PureLink Microbiome DNA Purification Kit include:

- Efficient lysis of all microorganisms (including durable species with thicker and more complex cell walls) by a combination of heat, chemical, and mechanical disruption with specialized beads
- Elimination of inhibitory compounds by precipitation using a proprietary cleanup buffer
- Streamlined protocols for numerous types of biological samples
- Recovery of highly pure DNA compatible with common downstream applications, such as qPCR and next-generation sequencing

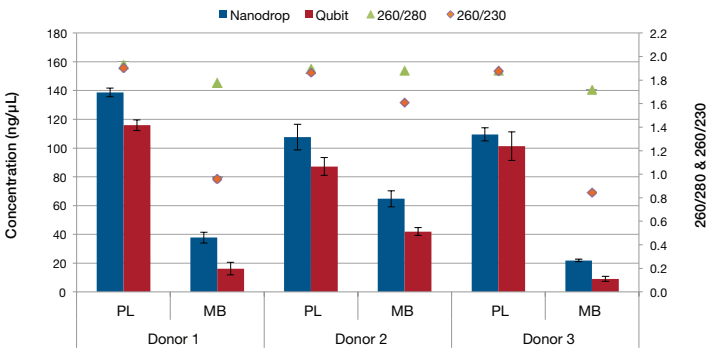
Analysis of microbial DNA in human stool samples

Within the human body, microbes reside in digestive and urogenital tracts, on the skin surface, the linings of the nasal passages, oral cavity, eyes, lungs, and mammary glands. The digestive tract, or gut, is the best-studied part of the human body in regard to its microbiome community, and it contains the largest and most diverse ecosystem compared to other sites. For example, the microbial community in the human large intestine contains 10^{11} to 10^{12} cells per milliliter of fluid. Human stool is the most widely used sample in studies of the microbiome, as it contains a large number of bacteria, fungi, and viruses that originate directly from the most dense microbial community of the human body.

Figure 1 highlights purification and subsequent qPCR analysis of microbial and host DNA from human stool. DNA was isolated from 0.2 g stool samples (in triplicates) obtained from three donors (D1–D3), using the PureLink Microbiome DNA Purification Kit and a competitor kit.

The concentration of DNA was measured using a NanoDrop spectrophotometer and a Qubit fluorometer, and DNA purity was determined by absorbance ratios using a NanoDrop spectrophotometer. The PureLink Microbiome DNA Purification Kit typically recovers 2–5 times more highly pure DNA versus the leading competitor's kit. Agarose gel analysis confirms a high level of DNA integrity and purity. qPCR analysis of three bacterial targets (*Bifidobacterium*, *Escherichia coli*, *Bacteroides/Prevotella*) with corresponding TaqMan Assays demonstrates that the samples produced with the PureLink Microbiome DNA Purification Kit display lower cycle threshold (C_t) values versus the competitor's kit—indicating better PCR amplification. Both abundant DNA template and low levels of inhibitors contribute to efficient PCR amplification. Similar results were obtained in several separate experiments with larger cohorts totaling 20 stool donors.

A



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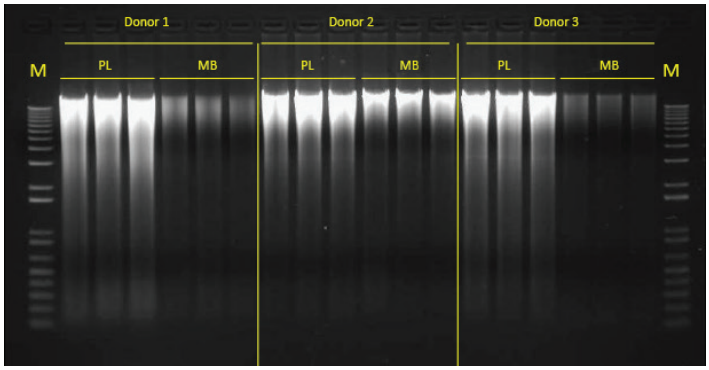

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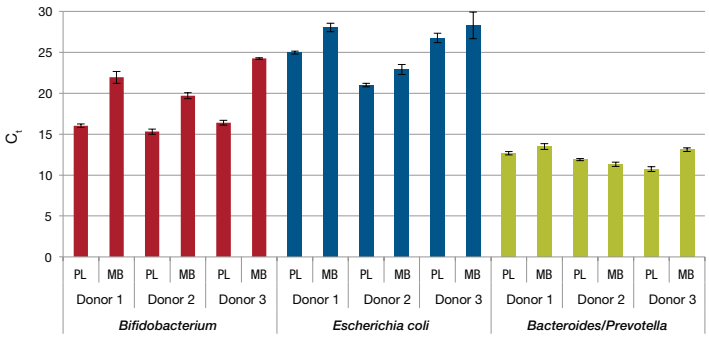

Figure 1. Purification of microbial and host DNA from human stool. DNA was isolated from 0.2 g stool samples (in triplicate) obtained from three donors (D1–D3), using the PureLink Microbiome DNA Purification Kit or a competitor's kit (kit MB). **(A)** Concentration of DNA, as measured using a NanoDrop spectrophotometer and a Qubit fluorometer, and DNA purity (absorbance ratios at 260/230 nm and 260/280 nm). Elution volume was 100 µL for both kits. **(B)** Analysis of DNA on an 0.8% agarose gel. M = 1 kb ladder. **(C)** qPCR analysis of three bacterial targets, *Bifidobacterium*, *Escherichia coli*, and *Bacteroides/Prevotella*, with corresponding TaqMan Assays.

An example of sequencing analysis of stool-derived bacterial DNA is shown in Figure 2. Isolation of total DNA from the stool samples of two donors (D4, D5), as well as initial DNA analysis, was performed as described above. 16S rRNA gene sequencing and profiling was performed in triplicate. Donor 4 has a diet high in meat, whereas Donor 5 has a diet high in plant-derived foods.

At the genus level, both donors were found to have the highest representation of *Bacteroides* (over 20% for both) and *Faecalibacterium* (over 20% for both). However, for many types of bacteria, notable differences between the donors were observed. The following bacteria were present at substantially higher levels in Donor 4: *Sutterella* (5.3–7% vs. <0.1% D5); *Coprococcus* (2.4–3.5% vs. 0.2–0.4% D5); *Streptococcus* (1.6–2.2% vs. <0.1% D5); *Clostridium* (2–2.8% vs. 0.3% D5).

The following bacteria were present at substantially higher levels in Donor 5: *Roseburia* (1.1–1.3% vs. 0.3–0.5% D4); *Lachnospira* (4.2–5.5% vs. 2–3.4% D4); *Subdoligranulum* (2.7–3.9% vs. <0.1% D4); *Ruminococcus* (2–2.3% vs. <0.1% D4); *Pseudobutyrivibrio* (13.6–21.4% vs. 5.5–6.2% D4).

The substantial differences observed between the stool microbiome communities of these two donors might be largely explained by differences in their diets. However it should be noted that the microbiome is also known to be affected by many other factors such as environment, health, uptake of antibiotics and probiotics, and age. The health aspect is extremely important, as the microbiome seems to influence (or at least is associated with) multiple diseases, including inflammatory bowel disease, malnutrition, celiac disease, obesity, vaginosis, asthma, diabetes, cancer, pancreatic disease, allergies, neurological disorders, and

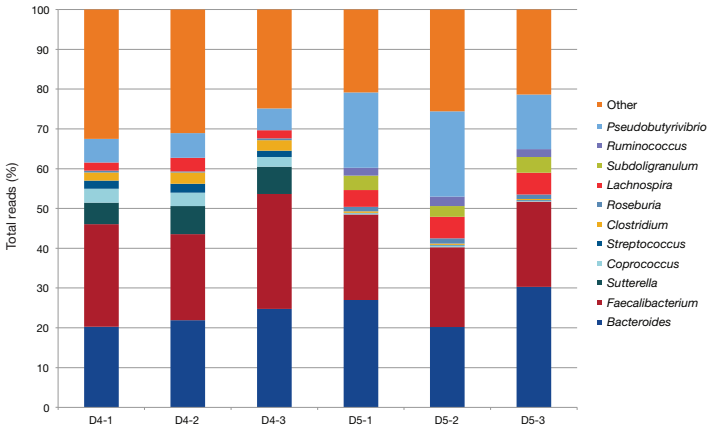

Figure 2. 16S rRNA gene sequencing analysis of bacterial DNA derived from human stool. Samples from two donors (D4, D5) were processed in triplicate. Representations of the most abundant 11 bacterial genera are shown as percentages of total reads.

heart disease. Although the specific tools described above are for Research Use Only and intended to aid fundamental research aimed at understanding the composition and functions of the microbiome community, the workflow itself may soon see practical application in diagnostics, monitoring efficiency of treatments, and detection of pathogens.

The apparent influence of the microbiome on broad aspects of human health has attracted an exponentially growing research effort, one that has the potential to revolutionize medicine. We developed complete workflow solutions for microbiome research, starting with sample collection and storage, and continuing to DNA isolation and downstream analysis. The new PureLink Microbiome DNA Purification Kit is central to this workflow, enabling fast purification of inhibitor-free microbial and host DNA from a wide variety of sample types, including particularly challenging samples such as human stool.

Find out more at **thermofisher.com/microbiome**

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