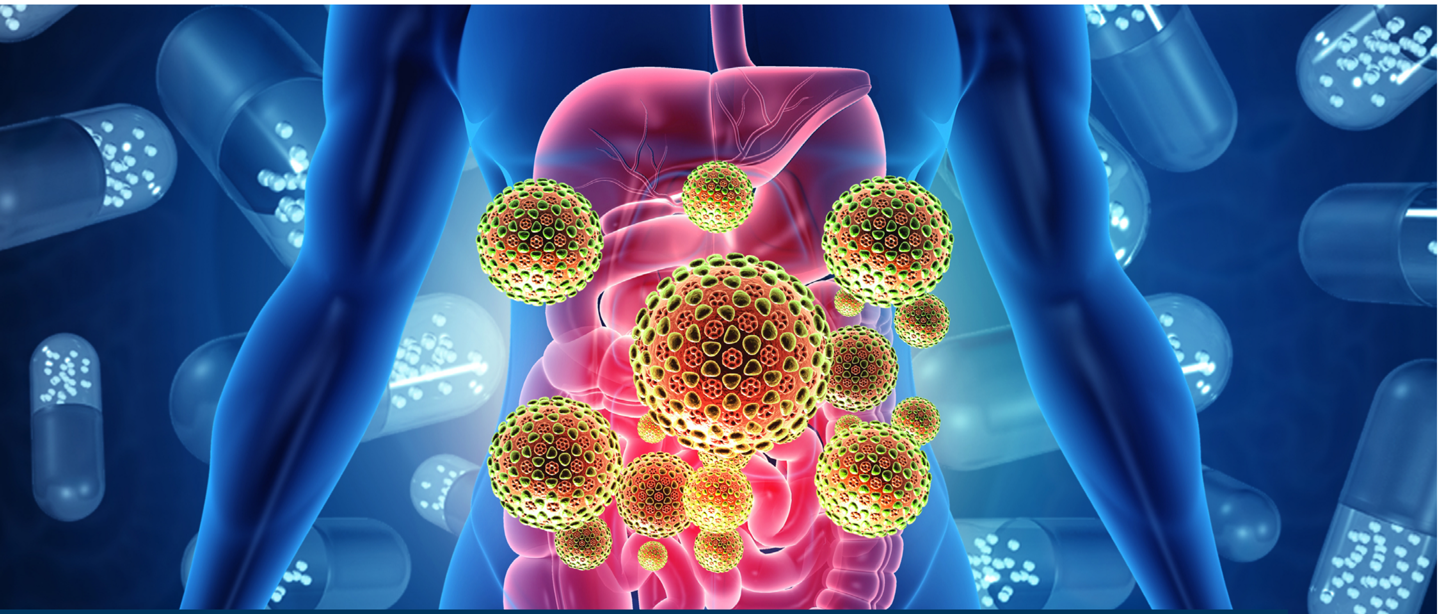


Intestinal Microbiome Analysis

The 16S Microbiome + ITS NGS Assay

A powerful end-to-end solution combining library preparation and bioinformatic analysis

Understanding the human microbiome

The human microbiome consists of a complex ecosystem of bacteria, fungi and viruses in numbers equal to the number of cells in the human body. It inhabits nearly every surface of our body, including the gastrointestinal tract, saliva, skin, conjunctiva and oral mucosa; and plays an essential role in health and disease.[1] Among these habitats, the gut microbiome is especially significant, influencing nutrient breakdown, vitamin synthesis, immune regulation, or communication with the nervous system.[2-4]

The importance of testing

By being involved in key biological processes (e.g., glucose metabolism, control of appetite and satiety via hormone regulation, prevention of pathogen invasion),[3-6] maintaining a balanced gut ecosystem is essential. In fact, an imbalance in microbial communities, known as dysbiosis, has been linked to various conditions ranging from obesity, type II diabetes, inflammatory bowel disease, and fatty liver disease to asthma, depression, and neurological disorders (e.g., autism, multiple sclerosis).[7-12] Understanding differences in bacterial and fungal communities across individuals, populations, health conditions, and geographical locations provides insights into how factors like diet, medication, or lifestyle shape this ecosystem.

Our approach

Targeted amplification of the **V3-V4 regions** of the 16S rRNA gene and the **ITS2 region** provides a quick and reliable way to profile the human gut microbiome. The V3-V4 regions capture variability between bacterial species for accurate diversity analysis, while the ITS2 region serves as a trusted molecular barcode for fungal identification and classification.[13] The 16S Microbiome + ITS NGS Assay, together with the Microbiome Analysis Webtool, provides a complete solution for species-level classification of bacteria and fungi colonizing the human gut.

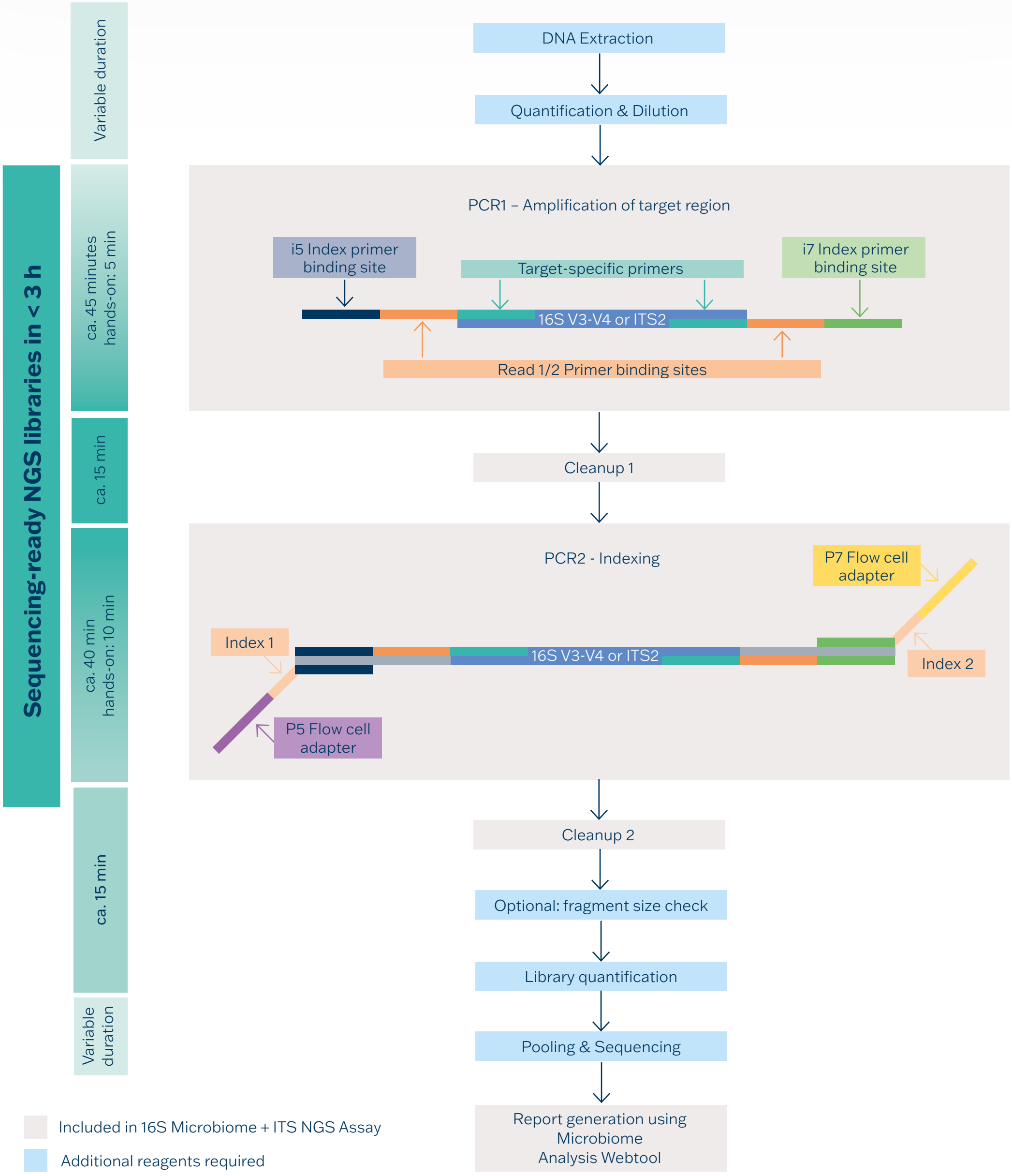
BioVendor
Group
NGS

Key Features

- Targeted library preparation and bioinformatic analysis
- Species-level classification of bacteria and fungi from as little as 25 ng of input DNA*
- End-to-end solution from extracted DNA to ready-to-sequence libraries in < 3 hours
- Flexibility with ITS2 primers supplied as an add-on
- Balanced heterogeneity spacers to increase library complexity for improved sequencing quality
- Dual-indexed libraries allow large-scale pooling (up to 288 samples)
- Illumina MiSeq compatible: fulfills the minimum 250 bp read length requirement
- Beta-diversity analysis down to genus-level
- Easy visualization: Krona interactive graphs, pie charts, PCoA plots and heatmap

*Limit of Detection: Fungi 10 pg in ≤ 10 ng bacterial/human DNA | Bacteria 2.5–25 ng input DNA.

16S Microbiome + ITS NGS Assay Workflow



Sequencing-ready NGS libraries in < 3 h

Variable duration

ca. 45 minutes
hands-on: 5 min

ca. 15 min

ca. 40 min
hands-on: 10 min

ca. 15 min

Variable duration

DNA Extraction

Quantification & Dilution

PCR1 – Amplification of target region

Cleanup 1

PCR2 - Indexing

Cleanup 2

Optional: fragment size check

Library quantification

Pooling & Sequencing

Report generation using
Microbiome
Analysis Webtool

⬜ Included in 16S Microbiome + ITS NGS Assay

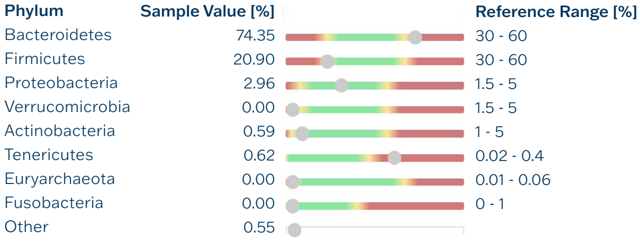
🟦 Additional reagents required

Disclaimer:
The 16S Microbiome + ITS NGS Assay enables precise identification of bacterial and fungal species by targeting the V3-V4 and ITS2 regions, using reference sequences from established databases. In principle, the assay can be used with any input sample containing high-quality bacterial and fungal DNA in sufficient quantities (e.g., DNA from urine, vaginal fluid, saliva). Currently, the reference ranges and explanatory content provided in the PDF report are tailored to the gut microbiome analysis.

Reporting using the Microbiome Analysis Webtool

A

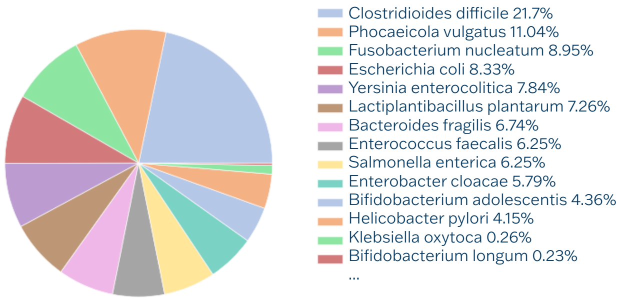
Bacterial Phyla



Phylum	Sample Value [%]	Reference Range [%]
Bacteroidetes	74.35	30 - 60
Firmicutes	20.90	30 - 60
Proteobacteria	2.96	1.5 - 5
Verrucomicrobia	0.00	1.5 - 5
Actinobacteria	0.59	1 - 5
Tenericutes	0.62	0.02 - 0.4
Euryarchaeota	0.00	0.01 - 0.06
Fusobacteria	0.00	0 - 1
Other	0.55	

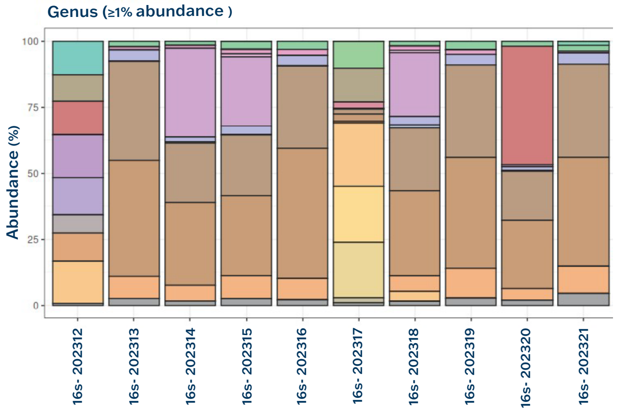
B

Proportion of QC passing reads Species



C

Genus Abundance



16S Microbiome + ITS Analysis Webtool

A. Bacterial composition of the individual sample at the phylum level compared to established reference ranges (green area of the color bar).

B. Bacterial composition at different taxonomic levels including phylum/class/order/family/genus and species-level classifications. The fungal composition (not displayed) is offered including phylum/class/order/family/genus.

C. Bar chart of genus abundance (≥1%) across samples, illustrating beta diversity and highlighting differences in microbial communities between groups, individuals, or treatment conditions.

The **Microbiome Analysis Webtool** is an easy-to-use platform providing an intuitive workflow for the bioinformatic analysis of 16S and ITS NGS data with clear and informative visualizations of the results.

Raw sequencing data (fastq files) is first uploaded into the Webtool and processed automatically. The pipeline integrates a sequence classification system in combination with the SILVA high-quality rRNA database and UNITE for the eukaryotic ITS region database.[14-16] For a comprehensive understanding of microbial communities, the pipeline delivers **species-level abundance data** in summary tables alongside graphical outputs such as pie charts and interactive Krona plots for rapid sample assessment.

In addition, the latest release introduces advanced **beta diversity analysis**, measuring differences in species composition across samples. Principal Coordinate Analysis (PCoA) plots, abundance charts and heatmap enabling comparison and visualization both within and between runs is now also possible. All results are compiled in detailed reports, simplifying the interpretation of microbial differences with visually meaningful results.

Each analysis delivers results in two formats: the **abundance data in raw format** for flexible downstream analysis, and customizable **human gut-tailored PDF reports** with concise summaries and personalization options.

References:

[1] Sender R et al. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016, 14(8):e1002533; [2] Tarracchini C et al. Exploring the vitamin biosynthesis landscape of the human gut microbiota. mSystems. 2024, 9(10):e0092924; [3] Wiertsema SP et al. The Interplay between the Gut Microbiome and the Immune System in the Context of Infectious Diseases throughout Life and the Role of Nutrition in Optimizing Treatment Strategies. Nutrients. 2021, 13(3):886; [4] Cryan JF et al. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019, 99(4):1877-2013; [5] Roubalová R et al. Influence of the gut microbiome on appetite-regulating neuropeptides in the hypothalamus: Insight from conventional, antibiotic-treated, and germ-free mouse models of anorexia nervosa. Neurobiol Dis. 2024, 195:106316; [6] Takiishi T et al. Intestinal barrier and gut microbiota: Shaping our immune responses throughout life. Tissue Barriers. 2017, 5(4):e1373208; [7] Turnbaugh PJ et al. A core gut microbiome in obese and lean twins. Nature. 2009, 457(7228):480-484; [8] de Souza HS et al. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol. 2016, 13(1):13-27; [9] Qin J et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012, 490(7418):55-60; [10] Abu-Shanab A et al. The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol. 2010, 7(12):691-701; [11] Hsiao EY et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell. 2013, 155(7):1451-1463; [12] Kho ZY et al. The Human Gut Microbiome - A Potential Controller of Wellness and Disease. Front Microbiol. 2018, 9:1835; [13] Nilsson RH et al. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol. 2019, 17(2):95-109; [14] Ounit R et al. CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. BMC Genomics. 2015, 16:236; [15] Quast C et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013, 41(D1):590-596; [16] Abarenkov K et al. The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. Nucleic Acids Res. 2024, 52(D1):D791-D797.

Kit components and order information

16S Microbiome + ITS NGS Assay

Library Preparation Kit for 16 and 96 libraries

Kit components		**[REF 9-131]**		**RUO**
Activation code		100 bioinformatic analyses		
Magnetic Beads		1x 13ml		
Box “Master Mix”	⚫	5x 1ml 16S Master Mix 2x		
Box “PCR1 V3-V4 + ITS2 Primer Mixes”	🟡	1x 400µl MIB1 - V3V4 Mix	🟢	1x 400µl FUN1 - ITS2 Mix
Box “PCR2 Indexing Forward Primers **Set A**”	🔵	8x 30µl MIB2A-F1 – MIB2A-F8 Primer		
Box “PCR2 Indexing Reverse Primers 1/2”	🟣	8x 30µl MIB2X-R1 – MIB2X-R8 Primer		
Box “PCR2 Indexing Reverse Primers 2/2”	🟣	4x 30µl MIB2X-R9 – MIB2X-R12 Primer		

Kit components		**[REF 9-132]**		**RUO**
Activation code		100 bioinformatic analyses		
Magnetic Beads		1x 13ml		
Box “Master Mix”	⚫	5x 1ml 16S Master Mix 2x		
Box “PCR1 V3-V4 + ITS2 Primer Mixes”	🟡	1x 400µl MIB1 - V3V4 Mix	🟢	1x 400µl FUN1 - ITS2 Mix
Box “PCR2 Indexing Forward Primers **Set B**”	🔵	8x 30µl MIB2B-F9 – MIB2B-F16 Primer		
Box “PCR2 Indexing Reverse Primers 1/2”	🟣	8x 30µl MIB2X-R1 – MIB2X-R8 Primer		
Box “PCR2 Indexing Reverse Primers 2/2”	🟣	4x 30µl MIB2X-R9 – MIB2X-R12 Primer		

Kit components		**[REF 9-133]**		**RUO**
Activation code		100 bioinformatic analyses		
Magnetic Beads		1x 13ml		
Box “Master Mix”	⚫	5x 1ml 16S Master Mix 2x		
Box “PCR1 V3-V4 + ITS2 Primer Mixes”	🟡	1x 400µl MIB1 - V3V4 Mix	🟢	1x 400µl FUN1 - ITS2 Mix
Box “PCR2 Indexing Forward Primers **Set C**”	🔵	8x 30µl MIB2C-F17 – MIB2C-F24 Primer		
Box “PCR2 Indexing Reverse Primers 1/2”	🟣	8x 30µl MIB2X-R1 – MIB2X-R8 Primer		
Box “PCR2 Indexing Reverse Primers 2/2”	🟣	4x 30µl MIB2X-R9 – MIB2X-R12 Primer		

Kit components		**[REF 9-131-16]**		**RUO**
Activation code		16 bioinformatic analyses		
Box "Magnetic Beads"		2x 1.25ml		
Box “Master Mix + PCR1 Primers”	⚫	1x 1ml 16S Master Mix 2x	🟡	1x 400µl MIB1 - V3V4 Mix
	🟢	1x 400µl FUN1 - ITS2 Mix		
Box “PCR2 Indexing Primers”	🔵	4x 30µl MIB2A-F1 – MIB2A-F4	🟣	4x 30µl MIB2X-R1 – MIB2X-R4

Note: REF 9-131, 9-132, 9-133 differ only in Indexing Primer Sets. If more than 96 samples are to be pooled on as single flow cell, it is recommended to order different index kit sets (e.g. Set A / REF 9-131 and Set B / REF 9-132). Ensure that each library in a pool uses a unique combination of indexing primers.
Attention: Indexing primers of REF 9-131-16 overlap with REF 9-131 Set A.

MANUFACTURER:

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