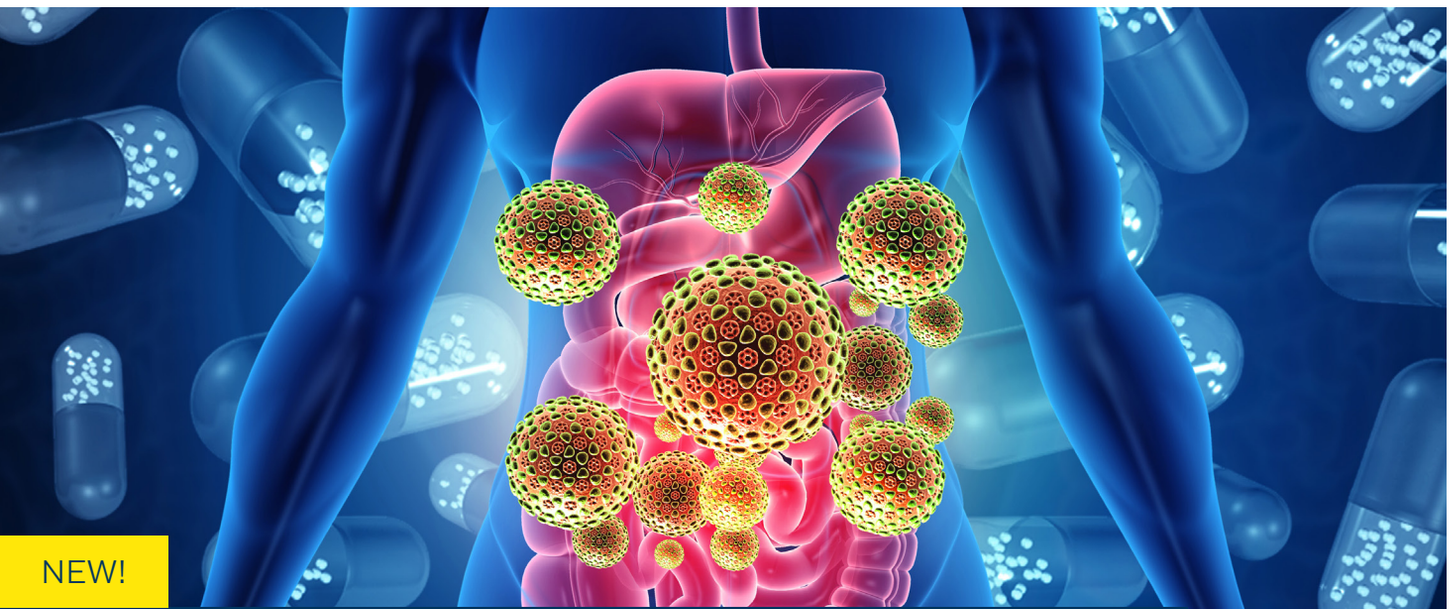


NEW!

Intestinal microbiome analysis

The 16S Microbiome + ITS NGS Assay

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

Intestinal microbiome

The human microbiome consists of a plethora 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 important role in health and disease[1].

Why testing?

Imbalances in the bacterial composition of the human gut have been linked to a wide range of conditions, including obesity, inflammatory bowel disease (IBD), type II diabetes, fatty liver disease, asthma, neurological disorders (e.g. autism, multiple sclerosis), mood disorders, and depression.[2-7]

Growing evidence suggests that fungi, although less common than bacteria, also play an important role in the human body. They have been shown to be involved in establishing the immune response and have been linked to IBD.[8]

What is being investigated?

Targeted amplification of the V3-V4 variable regions of the 16S rRNA gene and the ITS2 region provides a quick and easy way to assess the microbial composition of the human intestine. The V3 and V4 regions jointly bear the highest variability between different bacterial species rendering these regions well-suited for the in-depth analysis of bacterial diversity and composition. The same is true for the ITS2 region, which has been proposed as a molecular barcode for fungal classification. The ITS2 region is more suitable for unbiased taxonomic classification than the ITS1 region due to its lower length variation and more universal primer sites.[9] The ViennaLab 16S Microbiome + ITS NGS Assay in combination with the ViennaLab Microbiome Analysis Webtool enables for species-level classification of bacteria and fungi colonizing the human gut.

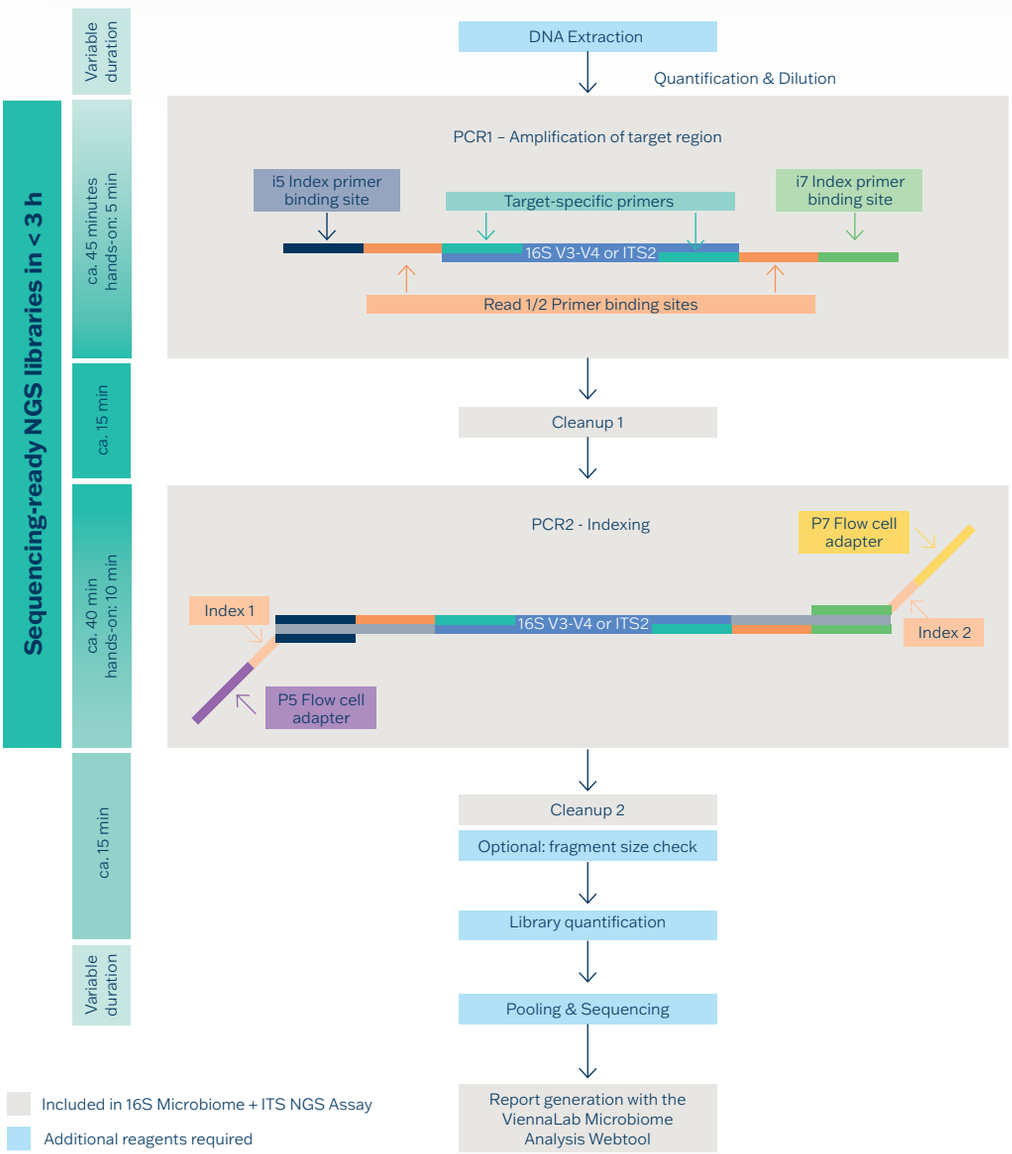
BioVendor Group NGS

Key features

- Targeted library preparation and bioinformatic analysis
- Species-level classification of bacteria and fungi in the human gut from as little as 25 ng of input DNA*
- From extracted bacterial and fungal DNA to ready-to-sequence libraries in < 3 hours
- ITS2 primers supplied as an add-on for user flexibility
- Balanced heterogeneity spacers to increase library complexity for improved sequencing quality
- Dual-indexed libraries allow large-scale pooling (up to 288 samples)
- Environmentally friendly kit configuration: fewer tubes → less waste
- Compatible with the Illumina MiSeq platform (fulfills the minimum 250 bp read length requirement)

*The limit of detection for fungal DNA is 10pg in up to 10ng of bacterial/human DNA. When testing bacterial composition only, the recommended input amount of DNA is 2.5 to 25 ng.

Workflow of the ViennaLab 16S Microbiome + ITS NGS Assay



Disclaimer:
The 16S Microbiome + ITS NGS Assay enables the analysis of bacterial and fungal species that can be differentiated through the V3V4 and ITS2 regions, for which their reference sequences are available in 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.

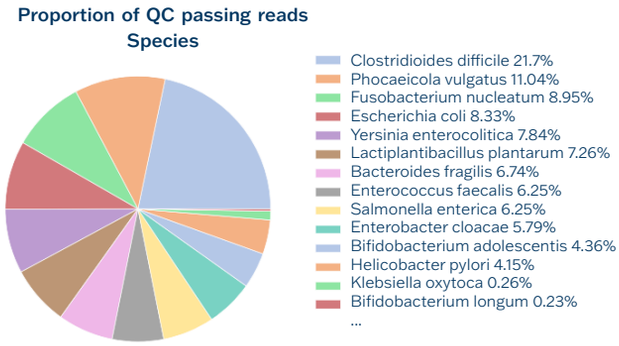
Reporting with the ViennaLab Microbiome Analysis Webtool

A

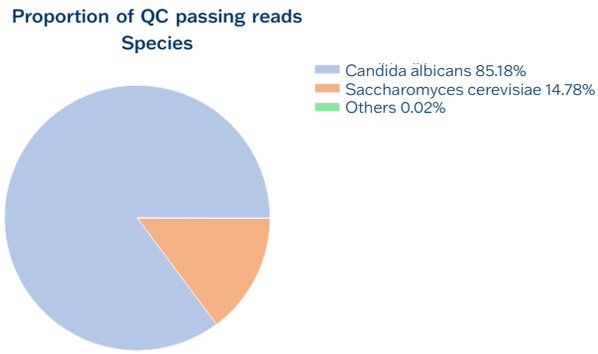
Bacterial Phyla

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



C



ViennaLab 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 (shown).

C. Fungal composition at different taxonomic levels including phylum/class/order/family/genus and species-level classifications (shown).

The ViennaLab **Microbiome Analysis Webtool** is an easy-to-use solution that provides an intuitive workflow for the bioinformatic analysis of 16S and ITS NGS data and the informative visualization of the computed classification results.

Raw sequencing data files can be uploaded directly to the Webtool and are automatically analyzed by an optimized pipeline using the CLARK sequence classification system in combination with the SILVA high-quality rRNA database and UNITE for the eukaryotic nuclear ribosomal ITS region database.[10-12]

The generated taxonomic output follows the NCBI classification nomenclature for easy cross-referencing. In addition, the pipeline provides diversity statistics based on species-level abundance, informative summary tables, and graphical output to facilitate the rapid review and comprehensive assessment of the microbial composition of the samples analyzed.

For each sample, a **human gut-tailored report in PDF format** is available for download. The report consists of the findings summarized and presented in a concise manner. Users have a possibility to personalise the report by adding their **institution's logo.**

Investigators can also download the species abundance data in a **raw format** and perform downstream analysis according to their specific needs.

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.
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[4] Qin J et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012, 490(7418):55-60.
[5] Abu-Shanab A, Quigley EM. The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2010, 7(12):691-701.
[6] Hsiao EY et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013, 155(7):1451-63.
[7] Kho ZY, Lal SK. The Human Gut Microbiome - A Potential Controller of Wellness and Disease. Front Microbiol. 2018, 9:1835.
[8] Pérez JC. Fungi of the human gut microbiota: Roles and significance. Int J Med Microbiol. 2021, 311(3):151490.
[9] Nilsson RH et al. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol. 2019, 17(2):95-109.
[10] Ounit R et al. CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. BMC Genomics 2015, 16:236.
[11] Quast C et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013, 41(D1):590-6.
[12] 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 96 and 16 libraries

RUO

NEW!	Kit components	[REF 9-131]
	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

NEW!	Kit components	[REF 9-132]
	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

NEW!	Kit components	[REF 9-133]
	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

NEW!	Kit components	[REF 9-131-16]
	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 you plan to sequence more than 96 samples on one flow cell, please order kits with different sets (e.g. Set A / REF 9-131 and Set B / REF 9-132). Make sure that all individual libraries sequenced in the same pool have a unique indexing primer combination.
Attention: Indexing primers of REF 9-131-16 overlap with REF 9-131 Set A.

MANUFACTURER:

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