AUTUMN INSPIRES NEW IDEAS!

Our autumnal offers with our flexible voucher option, our all-in-one package and flexible service. At StarSEQ we know that every project is unique.

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1-Step 16S and ITS Microbiome analysis promotion and voucher option

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1-Step Bacterial microbiome analysis or 1-Step Fungal microbiome analysis:

16S V4 region, Primer combination: 515F – 806R[1]
16S V4-V5 region, Primer combination: 515F – 909R[1]
16S V3-V4 region, Primer combination: 341F – 806R[4]
ITS ITS 1 region, Primer combination: ITS1F – ITS2[2]

Price example:

Package A: 288 samples only 26,52 € per sample and one region
Package B: 192 samples only 31,54 € per sample and one region
Package C: 96 samples only 47,08 € per sample and one region
Package D: 48 samples only 76,17 € per sample and one region

Price examples, all other sample sizes are possible. We charge per sample. The price is a combination of a fixed price for the run and a variable price for the libraries depending on the number of samples.
No shared runs, customer samples are sequenced exclusively on one MiSeq run!

Eukaryotes (microbial) analysis:

V8-V9 region, Primer combination: 18S-1422f (5'-ataacaggtctgtgatgccct-3') – 18S_1797R (5'-ccttcygcaggttcacctac-3')[5]
V9 region, Primer combination: Illumina_Euk_1391f (5'-GTACACACCGCCCGTC-3') – Illumina_EukBr_1510r (5'-TGATCCTTCTGCAGGTTCACCTAC-3'), blocking primer optional[3];
V9 region samples could not be combined with ITS, V8-V9 or 16S samples and need to be sequenced in a seperate MiSeq run because of smaller amplicon size!

Send us your gDNA samples and make use of our "All in One" service:

- **Quality control**
- **Single step amplicon generation with reduced bias**
- **Double indexing, quality check, quantification, normalization and pooling of amplicons**
- **Illumina MiSeq / NextSeq 2000™ sequencing package A-D: 2 x 300 nt paired-end sequencing with V3 chemistry**
- **Output package A-D: 20-30 M reads (including 25 % PhiX to balance the composition of bases)**
- **De-multiplexing of reads**
- **Data delivery via FTP server**

[1,2,3,4,5] Primer sequence references: Please see page 4!

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2-Step 16S and ITS Microbiome analysis

2-Step Bacterial and Archaea Microbiome analysis:

16S, V1-V2 region, Primer combination 27F (5'-AGAGTTTGATCMTGGCTCAG-3') – 338R (5'-TGCTGCCTCCCGTAGGAGT-3')

2-Step Fungal (18S) microbiome analysis:

ITS, ITS 1/2 region, Primer combination: ITS-u2 (5'-GAAYCATCGARTCTTTGAACGC-3') – ITS-p4 (5'-CCGCTTAKTGATATGCTTAAA-3')

ITS, ITS 2 region, Primer combination: ITS3F (5'-GCATCGATGAAGAACGCAGC-3') – ITS4R (5'-TCCTCCGCTTATTGATATGC-3')

2-Step Barcoding and Custom Amplicon analysis

2-Step Barcoding:

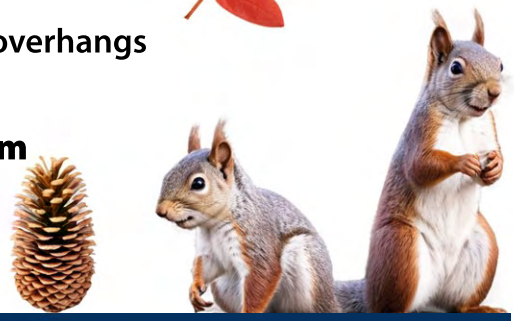
cytochrome b, cytb1 (5'-ccatccaacatctcagcatgatgaaa-3') – cytb2 (5'-gcccctcagaatgatatttgtcctca-3')

cytochrome oxidase I, CO I E (5'-ccagagattagagggaatcagtg-3') – CO 1 F (5'-cctgcaggaggaggagaycc-3')

or 2-Step Custom Amplicons:

design of custom primers with Illumina specific overhangs

Request for offer: microbiome@starseq.com



Optional: gold standard bioinformatic analysis of 16S/18S/ITS sequences (QIIME2)

The bioinformatics analysis pipeline consists of quality control, pre-processing of reads, taxonomy identification and visualization, calculation of alpha and beta-diversity metrics.

Request for offer: microbiome@starseq.com

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16S and ITS Microbiome analysis

Sequencing of your ready to load 16S/ITS/18S libraries

- Illumina MiSeq / NextSeq 2000™ sequencing: 2 x 300 nt paired-end sequencing with V3 chemistry
- Output 20-30 M reads (including 25 % PhiX to balance the composition of bases)
- De-multiplexing of reads
- Free 16S metagenomics analysis (Illumina App)
- Data delivery via FTP server

Only 3300 € for MiSeq sequencing

Optimize your research with our 16S/ITS workflow solutions

From sample prep to library prep to sequencing and bioinformatics. StarSEQ offers a complete and comprehensive workflow for various 16S/ITS applications. Sequencing of the hypervariable regions of the 16S rRNA gene is the gold-standard for identification and classification of bacterial communities within a given sample.

StarSEQ is using established gold-standard primer sequences to target regions covering the hypervariable regions V3-V4, V4 and V4-V5. In contrast to common used methods, StarSEQ has designed its own single-step technique for simultaneous amplification, indexing and adaptoring of samples to reduce the bias caused during PCR.

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16S and ITS Microbiome analysis

Applications:

- Environmental Metagenomics (soil, water, air, biofilms, complex organic communities)
- Human or Animal Microbiome (skin, stool, gut, blood, swab)
- Monitoring of animal health (e.g. alternatively or supplementarily to FELASA-Test)
- Sterility Monitoring
- Detection of Contamination
- Monitoring of Biogas Plant
- Biosafety Monitoring
- Food Quality
- Clinical Samples . . .

You have no time/staff for routine preparation of gDNA from your collected samples?

As an additional service we offer also the purification of gDNA starting from 18 €.

Primer sequence references:

[1] Apprill A, McNally S, Parsons R, Weber L. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol. 2016;18: 1403-1414.

[1] Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology, 18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

[1] Walters, W., Hyde, E. R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., … Knight, R. (2016). Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. *mSystems, 1*(1), e00009-15. https://doi.org/10.1128/mSystems.00009-15

[2] White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, NY. Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113-118.

[3] Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W., & Huse, S. M. (2009). A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes. *PLOS ONE, 4*(7), e6372. Retrieved from https://doi.org/10.1371/journal.pone.0006372

[4] Klindworth A., Pruesse E., Schweer T., Peplies J., Quast C., Horn M., et al. . (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, 1–11. 10.1093/nar/gks808
see also https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html

[5] Bradley, I. M., Pinto, A. J., & Guest, J. S. (2016). Design and Evaluation of Illumina MiSeq-Compatible, 18S rRNA Gene-Specific Primers for Improved Characterization of Mixed Phototrophic Communities. Applied and Environmental Microbiology, 82(19), 5878 LP-5891. https://doi.org/10.1128/AEM.01630-16

see also https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_preparation.html

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