

# Amplicon Metagenomics

## Sample Amounts and Concentrations

Amount and Concentration of DNA	Container
Provide total DNA ( $\geq 0.1 \mu\text{g}$ per sample with $\geq 5 \text{ ng}/\mu\text{l}$ ) in $\geq 20 \mu\text{l}$	1-24 samples: tubes or 96-well PCR plate >24 samples: 96-well PCR plate

### Remarks:

- If you would like to send samples for isolation, please refer to our user guide for DNA Isolation.
- DNA samples are preferably dissolved in DNase-free water or 10 mM Tris-HCl buffer (pH 7.5 – 8.5).
- DNA samples dissolved in the above buffers can be sent at ambient temperatures.

## Sample Preparation

When preparing your samples, please ensure that your samples are placed in low DNA/RNA affinity plates (PCR plates) and that they are properly sealed (heat sealed or 8-cap strips).

Use 1.5 ml tubes only for less than 24 samples. Screw-capped tubes are the most robust and secure tubes (no accidental lid opening). If you are using snap-cap tubes, we recommend that you use Safe-Lock/SafeSeal tubes (less risk of accidental lid opening).

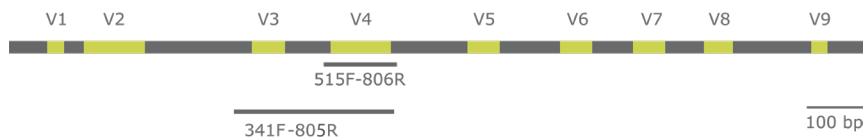


## Choice of the Primer System

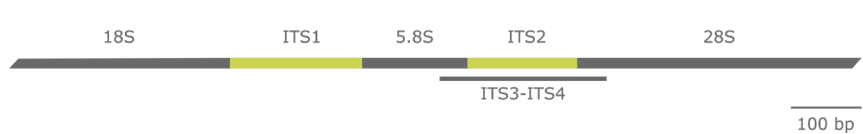
Amplicon metagenomics is based on NGS sequencing of the microbial rRNA gene. Since NGS read lengths are limited, only parts of the rRNA gene can be amplified and sequenced. For prokaryotes, the analysis targets hypervariable regions (V1-9) on the 16S rRNA gene, while for fungi the internal transcribed spacer regions (ITS) are used for taxonomic profiling (see **Figure 1**).

An ideal primer system should be universal enough to cover a broad range of taxonomic groups, while the resulting amplicon must provide enough taxonomic information for a reliable taxonomic classification. Based on our experience and the validation of our 16S/ITS analysis pipeline, we recommend the primer systems displayed in **Table 1**. Our service is not restricted to the displayed marker genes and primer systems, also other phylogenetic marker genes (e.g. cytochrome c oxidase I) and primer systems can be used. A pilot study can be very helpful to find the best primer system for your specific research question.

### A Prokaryotic 16S rRNA gene



### B Internal Transcribed Spacer Regions (ITS1 & ITS2)



**Figure 1.** Overview of loci of ribosomal gene loci commonly used for the taxonomic analysis of microbial communities. Hypervariable regions are marked in green while conserved regions are marked in grey.

A. Structure of the prokaryotic 16S rRNA gene showing the nine hypervariable regions (V1-V9) and the regions targeted by the commonly used primer systems. B. Organization of the fungal rRNA gene operon showing two internal transcribed spacer regions (ITS). ITS2 is most often used for profiling fungal communities.

Primer Name	Sequence (5'-3')	Region	Size (bp)	Source
515F (Parada) 806R (Apprill)	GTGYCAGCMGCCGCGGTAA GGACTACNVGGGTWTCTAAT	V4	~300	[1]
341F 805R	CCTACGGGNGGCWGCAG GACTACHVGGGTATCTAATCC	V34	~460	[2]
ITS3 ITS4	GCATCGATGAAGAACGCAGC TCCTCCGCTTATTGATATGC	ITS2	300-400	[3]

**Table 1.** Recommended and commonly used universal primer systems for prokaryotic (515F/806R, 341F/805R) and fungal (ITS3/ITS4) microbiome profiling. Only template specific sequences are shown. For Illumina-Sequencing the primer sequences must be combined with Illumina adaptor sequences in a two-step PCR approach. For further information consult our application note for Amplicon Deep Sequencing (<https://www.microsynth.com/amplicon-deep-sequencing.html>).

**Important:** For custom primer sets get in contact with your sales representative.

## Sample Preparation and Sequencing

Standard library preparation involves sample quality control and a two-step PCR amplification process using a Microsynth standard primer set specified upon sample registration. The resulting PCR products are then purified, analyzed by gel electrophoresis, quantified, and equimolarly pooled. If the first-round PCR fails, the sample preparation will be repeated. Sequencing is performed using convenient per-sample data packages, which consist of 100,000 forward and 100,000 reverse reads per sample. Alternatively, customized volume sequencing packages are available upon request.

## Bioinformatic Analysis

The standard bioinformatics analysis involves clustering reads into OTUs (zOTUs/amplicon sequence variants/ASVs), assigning taxonomic profiles, calculating  $\alpha$ -diversity, and generating rarefaction curves. Before clustering, the raw paired-end reads are merged, quality filtered, and checked for chimeras. Taxonomic assignment is provided at each level down to the genus level (and species level where feasible).

If technically possible, additional analysis modules can be added at extra cost:

### Comparative Statistics

The comparative statistics analysis builds on the amplicon metagenomics analysis and includes determination of  $\beta$ -diversity and differential OTU analysis based on replicates among the samples and on categories provided by the customer.

### Functional Profiling of 16S Amplicons

Prediction of the functional potential of the samples. Based on an analysis of operational taxonomic units (OTUs) using the 16S marker, taxa are identified and functional genetic information is inferred from surveys and full genetic information.

## **Sample Shipment / Order Form Completion**

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Prior to shipping your sequencing samples to Microsynth, please proceed as follows to complete your order form:

1. Enter our webshop at <https://srvweb.microsynth.ch/>
2. Click on „**Illumina Sequencing**“ and afterwards on „**Amplicon Metagenomics**“ in the green Analysis Services area
3. Fill in the order form and submit your order. Feel free to communicate any specific requirements or provide additional information using the comment field and file upload options.
4. Prepare your samples according to this User Guide or in case of isolation according to the DNA isolation User Guide.
5. If you have ordered DNA/RNA isolation along with NGS analysis, please select an appropriate Next Generation Sequencing entry point and select “Material for Isolation” for Sample Types.
6. Send your samples together with the printout to Microsynth AG according to the listed conditions, adding “NGS” or “Isolation” to the address.

## **Need More Information?**

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