CD Genomics
The Genomics Services Company

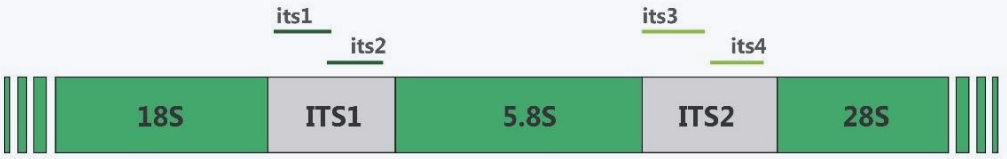
How to Choose the Best 16S/18S/ITS Sequencing Method for Your Project?

Overview

16S/18S/ITS amplicon sequencing is a prevalent method to identify taxonomic composition and complex microbial communities. The commonly used sequencing platforms include NGS platforms like Illumina MiSeqand and long-read platforms like Pacific Biosciences (PacBio) systems. Various PCR primers have been developed for this method. 16S/18S/ITS sequencing can be further subdivided into short-read, absolute quantitative, and full-length 16S/18S/ITS sequencing.

16S rRNA gene.

18S rRNA gene.



The internal transcribed spacer (ITS).

Short-read 16S/18S/ITS sequencing

Illumina MiSeqis is the most widely used platform for short-read 16S/18S/ITS sequencing. Its chemistry allows the approximately 300 bp PCR fragments to be sequenced in both directions. Short-read sequencing is appropriate to characterize single or two variable regions, such as V1-V2, V3-V4, V4, V4-V5 region of 16S rRNA gene using paired 300-bp reads. As the V3-V4 region is longer than 300 bp, the ends of each read are overlapped to generate full-length V3-V4 region.

Table 1. The strategies for NGS-based 16S/18S/ITS sequencing.

	Amplification Region	Sequencing Strategy
Bacteria	V1-V3 of 16S rDNA	PE300
	V3-V4 of 16S rDNA	PE300
	V4 of 16S rDNA	PE250
	V4-V5 of 16S rDNA	PE300
Eukaryotes	V4 of 18S rDNA	PE250
Fungi	ITS1	PE250
	ITS2	PE300

Table 2. The advantages and disadvantages of NGS-based 16S/18S/ITS sequencing.

Advantages	Disadvantages
◆ Can be applied to microbial diversity analysis, functional analysis, microbial phylogenetic profiling ◆ Able to assess relative abundance of subgroups ◆ High throughput and reliable results ◆ Cost-efficient and cost-effective	◆ Typically provides only family-or genus-level taxonomy ◆ Could not provide absolute abundance of subgroups

Absolute quantitative 16S/18S/ITS sequencing

The traditional 16S/18S/ITS sequencing method can only obtain the relative abundance data by measuring the number of sequences of a certain operational taxonomic unit (OTU) to the total sequence number ratio. Absolute quantitative sequencing using synthetic chimeric DNA spikes can be used to assess absolute abundance of subgroups in the complex microbial community. Adding a known amount of synthetic DNA spikes to environmental samples and calculating their relative abundance in the sequencing output enable the assessment of the absolute abundance for specific groups of the microorganisms (Figure 1).

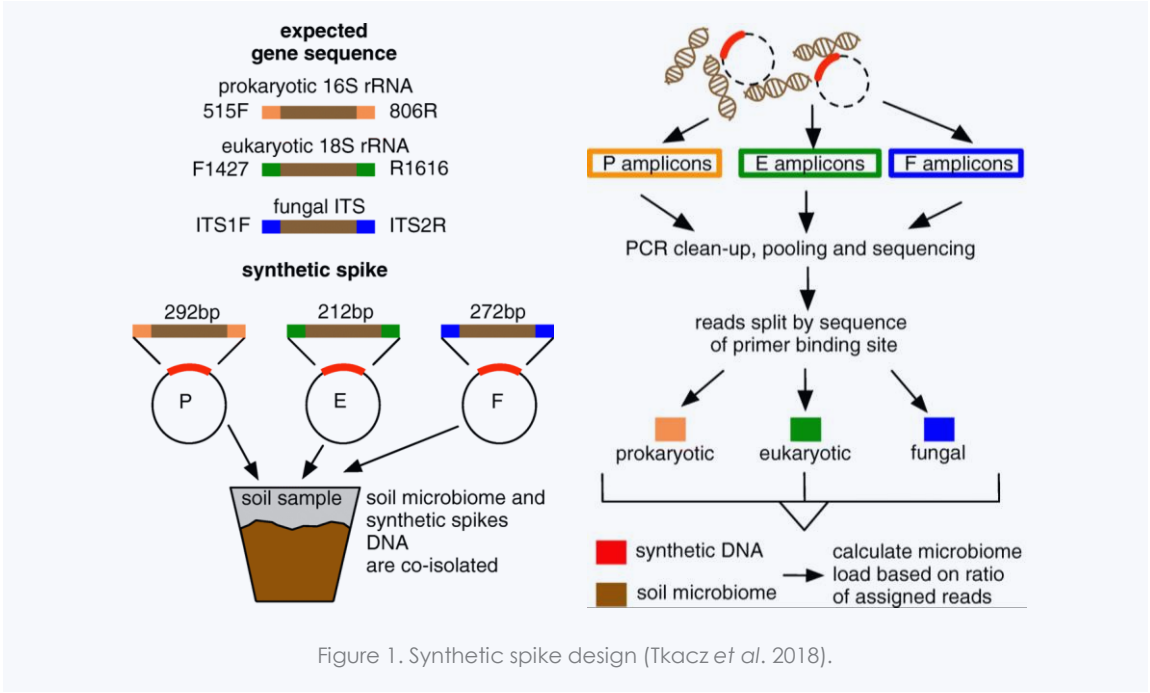


Figure 1. Synthetic spike design (Tkacz *et al*. 2018).

Table 3. The advantages and disadvantages of absolute quantitative 16S/18S/ITS sequencing.

Advantages	Disadvantages
◆ Can be applied to microbial diversity analysis, phylogenetic profiling, functional analysis ◆ Assess absolute abundance of subgroups ◆ High throughput and reliable results	◆ Typically provides only family-or genus-level taxonomy

Full-length 16S/18S/ITS sequencing

PacBio's long-read sequencing technology used to suffer from high error rates per base. Instead, the PacBio circular consensus sequencing (CCS) technology can generate highly accurate (99.8%) long reads with an average of 13.5 kb, not to mention the full-length 16S/18S/ITS. CCS enables the polymerase to repeatedly replicate the circularized strand and produces one long read with randomly distributed errors (Figure 2). Using PacBio CCS mode, microbiome analyses will provide high-fidelity species-level data.

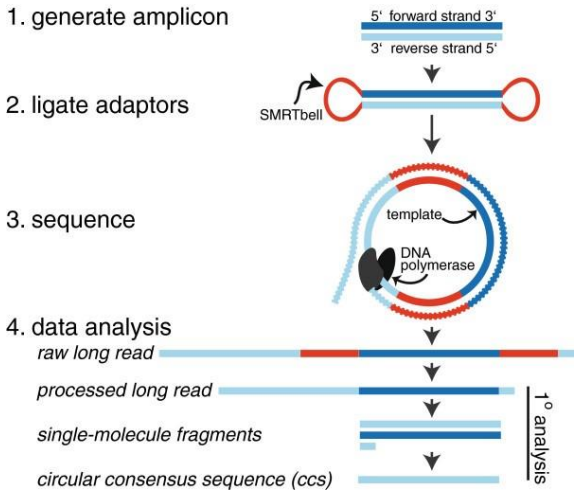


Figure 2. Illustration of PacBio sequence generation using CCS mode (Fichot & Norman 2013).

Table 4. The advantages and disadvantages of full-length 16S/18S/ITS sequencing.

Advantages	Disadvantages
◆ Multiple applications: microbial diversity analysis, evolutionary analysis, and functional analysis. ◆ High throughput and high-fidelity data ◆ Can distinguish among closely related organisms, even when sequence differences are insufficient to divide these into distinct OTUs ◆ Provides species-level microbiome data ◆ Assess absolute abundance of subgroups	◆ Relatively costly ◆ Relative reduced throughput ◆ Cannot provide absolute abundance of subgroups

Reference:

1. Tkacz A, Hortala M, Poole P S. Absolute quantitation of microbiota abundance in environmental samples. *Microbiome*, 2018, 6(1): 110.
2. de Boer P, Caspers M, Sanders J W, *et al*. Amplicon sequencing for the quantification of spoilage microbiota in complex foods including bacterial spores. *Microbiome*, 2015, 3(1): 30.
3. Wenger A M, Peluso P, Rowell W J, *et al*. Highly-accurate long-read sequencing improves variant detection and assembly of a human genome. *bioRxiv*, 2019: 519025.
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5. Fichot E B, Norman R S. Microbial phylogenetic profiling with the Pacific Biosciences sequencing platform. *Microbiome*, 2013, 1(1): 10.
6. Earl J P, Adappa N D, Krol J, *et al*. Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes. *Microbiome*, 2018, 6(1): 190.