

# 16S/18S/ITS Amplicon Sequencing

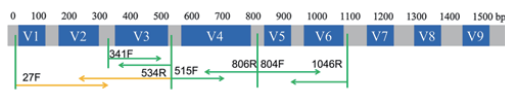


16S/18S/ITS amplicon sequencing aims at revealing phylogeny, taxonomy, and species abundance in a microbial community by investigating PCR products of housekeeping genetic markers that contain both highly conserved and hypervariable parts. The introduction of these perfect molecular fingerprint by Woese et al.(1977) empowers isolation-free microbiome profiling. Sequencing of 16S (bacteria), 18S (fungi) and Internal transcribed spacer(ITS, fungi) allows identification of both abundant species as well as rare and unidentified species. This technology has becoming a widely applied and major tool in identifying differential microbial composition in various environments, such as human mouth, intestines, feces, etc.

## NGS-based amplicon Seq

### For bacteria: 16S V3+V4

16S rDNA: 16S rRNA gene codes RNA component of 30S subunit. 16S rDNA consists of conserved regions indicating phylogeny information among species and hypervariable regions reflecting differences among species.

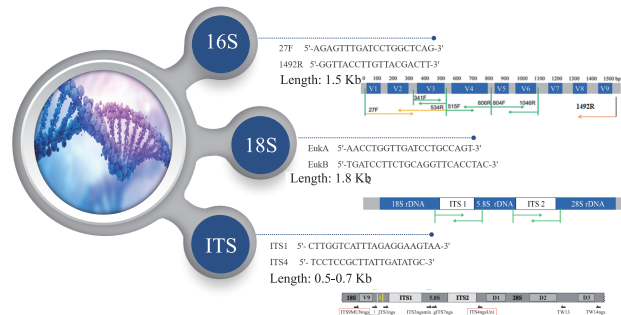


### For fungi: ITS1

ITS1: ITS1 located between 18S rDNA and 5.8S rDNA of eukaryotes. ITS1 undergoes 10 time more rapid concerted evolution compared to 18S rDNA.



## Full-length amplicon Seq

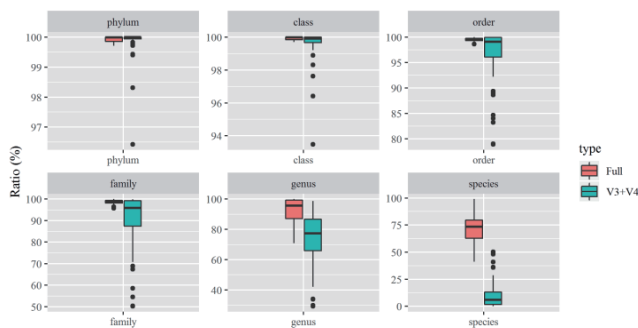


### PacBio-based Full-length amplicon sequencing

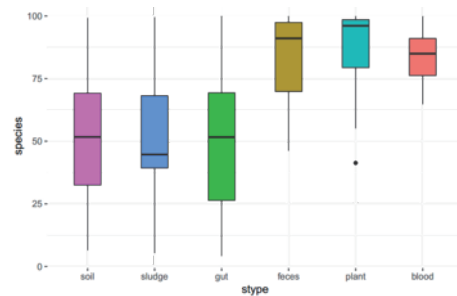
Single-molecule real-time(SMRT) sequencing of PacBio platform enables obtaining of highly accurate long reads, which could cover full-length amplicons (approx. 1.5 Kb). The wider view of genetic field greatly enhanced the resolution in species annotation in bacteria or fungi community.

## Service advantages

	NGS-based amplicon sequencing	Full-length amplicon sequencing
Sequencing platform	Illumina	PacBio; CCS mode: min Passes ≥ 5
Species annotation	“Genus” level	“Specie” level (average annotation rate: ≥ 60%)
Targeting region	V3+V4, ITS1	V1-V9; ITS1+ITS2
Data	Pair-end fastq	Single-end fastq (assembly free)
BMKCloud	Amplicon sequencing analyzing APP has been established and open for free trial on both standard and advanced analysis	



Annotation rate of V3+V4(Illumina)-based profiling vs. full-length(PacBio)-based profiling (Data of 30 randomly picked projects were applied for statistics.)

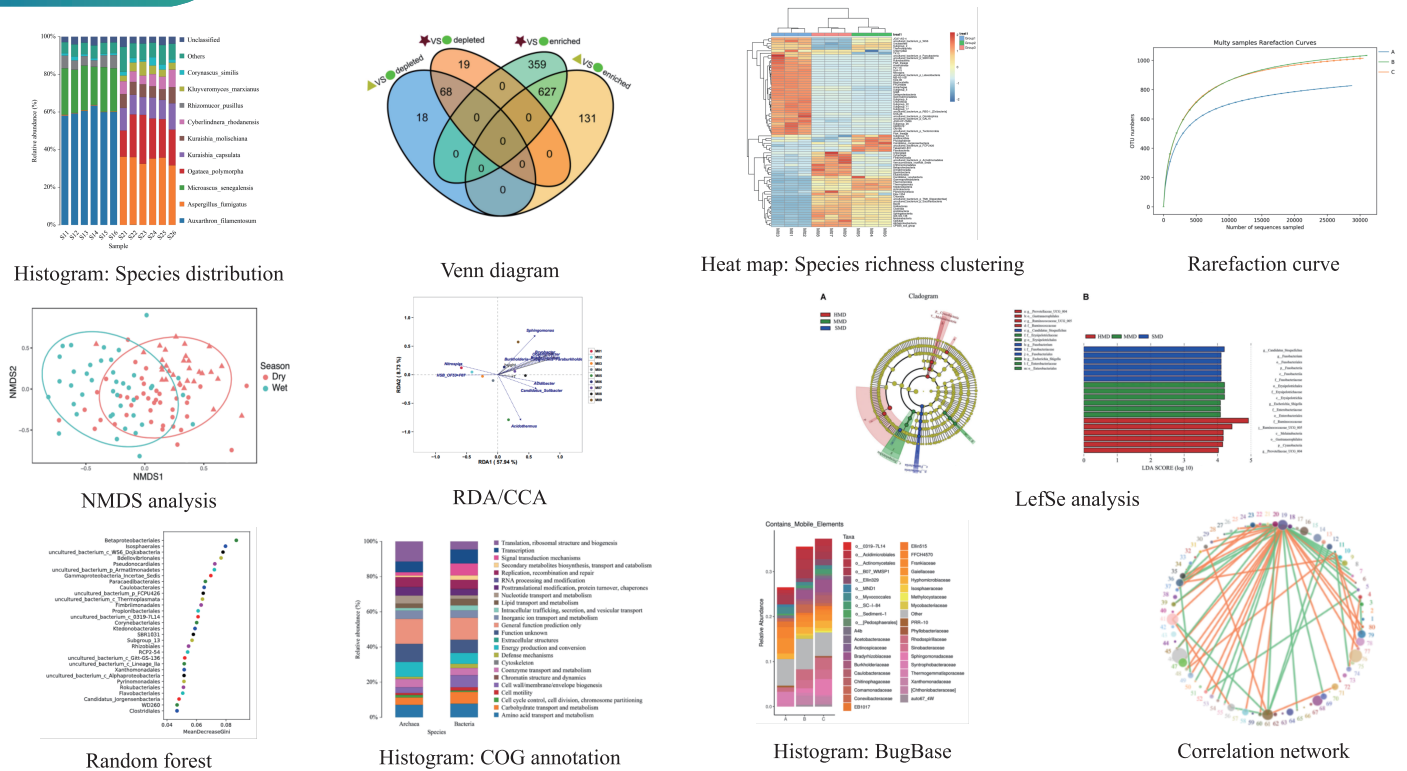


Annotation rate of full-length amplicon sequencing at species-level in different sample types.

## Service specifications

Service	Platform	Data volume	Turn-around Time
NGS amplicon sequencing	Illumina	Bacteria 16S V3+V4/Fungi ITS1: 50-300 K tags	30 working days
Full-length amplicon sequencing	PacBio	Bacteria V1-V9/Fungi ITS1+ITS2: 5-10 K CCS reads	30 working days

## Demo results



## Case study



### 16S rDNA sequencing and metagenomics revealing differences in gut microbiome composition

#### Title: Obese Individuals with and without Type 2 Diabetes show different gut microbial functional capacity and composition

**Background:** Obesity and Type 2 diabetes are both known to be associated with metabolic disorders caused by gut microbiome changes. Therefore, co-occurrence of both diseases makes it even more difficult to distinguish unique microbiome changes caused by either diseases.

**Material and methods:** Lean non-diabetes (n=633); Obese non-diabetes (n=494); Obese-Type 2 diabetes (n=153);

Target region: 16S rDNA V1-V2

Platform: Illumina Miseq (NGS-based amplicon sequencing)

Subset of DNA extracts were subjected to metagenomic sequencing on Illumina Hiseq

Main result: Microbial profilings of these metabolic diseases were successfully differentiated.

By comparing microbial features generated by 16S sequencing, obesity was found to associated with changes in microbial composition, individual features, especially significant decrease in Akkermansia, Faecalibacterium, Oscillibacter, Alistipes, etc. In addition, T2D was found associated with increase in Escherichia/shigella.

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