

16S/18S/ITS Amplicon Metagenomic Sequencing

1. Sample Requirements

Sample Type	Amount(Qubit®)	Concentration	Volume	Purity(NanoDrop™)
Total DNA	≥ 200 ng	≥ 10 ng/μL	≥ 20 μL	OD260/280=1.8~2.0 no degradation or contamination

2. Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Paired-end 250 bp
Recommended sequencing depth	30 kb/50 kb/100 kb raw reads
Data quality	Guaranteed ≥ 75% bases with Q30 or higher
*Turnaround time	Within 4 weeks from project verification to data releasing without bioinformatic analysis

*Turnaround time varies depending on the project volume.

3. Primer List of Amplicons

Types	Region	Fragment Length	Primer	Primer sequences (5' - 3')
Bacterial 16S	V4	300 bp	515F	GTGCCAGCMGCCGCGGTAA
			806R	GGACTACHVGGGTWTCTAAT
	V3-V4	470 bp	341F	CCTAYGGGRBGCASCAG
			806R	GGACTACNNGGTATCTAAT
	V4-V5	450 bp	515F	GTGCCAGCMGCCGCGGTAA
			907R	CCGTC AATTCCTTTGAGTTT
	V5-V7 (for endophytic)	300-400 bp	799F	AACMGATTAGATACCKG
1193R			ACGTCATCCCCACCTTCC	
Archaeal 16S	V4 -V5	400-500bp	Arch519F	CAGCCGCCGCGGTAA
			Arch915R	GTGCTCCCCGCCAATTCCT
Archaeal 1106F	V8	300bp	1106F	TTWAGTCAGGCAACGAGC
			1378R	TGTGCAAGGAGCAGGGAC
Fungal 18S	V4	350 bp	528F	GCGGTAATTCAGCTCCAA
			706R	AATCCRAGAATTCACCTCT
	V9	200 bp	1380F	CCCTGCCHTTTGTACACAC
			1510R	CCTTCYGCAGGTTACCTAC

Types	Region	Fragment Length	Primer	Primer sequences (5' - 3')
Fungal ITS	ITS1	200-400 bp	ITS5-1737F	GGAAGTAAAAGTCGTAACAAGG
			ITS2-2043R	GCTGCGTTCTTCATCGATGC
	ITS2	380 bp	ITS3-2024F	GCATCGATGAAGAACGCAGC
			ITS4-2409R	TCCTCCGCTTATTGATATGC
	ITS1-1F (for endophytic)	200-400 bp	ITS1-1F-F	CTTGTCATTTAGAGGAAGTAA
			ITS1-1F-R	GCTGCGTTCTTCATCGATGC

4. Data Analysis Contents

16S/18S/ITS Amplicon Metagenomic Sequencing Standard Analysis

Data quality control

OTUs cluster and phylogenetic relationship construction

Species annotation

Alpha diversity analysis (Observed species, Goods coverage, Chao1, ACE, Shannon, Simpson Index)

Beta diversity analysis (Unifrac distance heatmap, PCA, PCoA, UPGMA)

Ternaryplot

NMDS analysis

LefSE analysis

Metastats analysis

Species T-test analysis

MRPP, Anosim, Adonis, Amova analysis

Comparative analysis of alpha diversity indices

Comparative analysis of beta diversity indices

naryplot

16S/18S/ITS Amplicon Metagenomic Sequencing Advanced Analysis

Spearman, CCA/RDA, VPA analysis

Network analysis

PICRUSt analysis