16S/18S/ITS Amplicon Metagenomic Sequencing

1. Sample Requirements

Sample Type	Amount(Qubit®)	Concentration	Volume	Purity(NanoDrop™)
Genomic DNA	≥ 200ng	≥ 20 ng/μL	≥ 12 μL	OD260/280=1.8~2.0 no degradation or contamination

Remark: This sample requirement is for reference only. If you have any questions, please consult your local sales or Novogene support for detailed information.

2. Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Paired-end 250 bp
Sequencing depth	30 kb/50 kb/100 kb raw reads
Data quality	Guaranteed ≥ 75% bases with Q30 or higher

Remark: Detailed sequencing parameters can be consulted with your local sales or Novogene support.

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	GGACTACNNGGGTATCTAAT
	V4-V5	450 bp	515F	GTGCCAGCMGCCGCGGTAA
			907R	CCGTCAATTCCTTTGAGTTT
	V5-V7 (for endophytic)	300-400 bp	799F	AACMGGATTAGATACCCKG
			1193R	ACGTCATCCCCACCTTCC
Archaeal 16S	V4 -V5	400-500bp	Arch519F	CAGCCGCCGCGGTAA
			Arch915R	GTGCTCCCCCGCCAATTCCT
Archaeal 1106F	V8	300bp	1106F	TTWAGTCAGGCAACGAGC
			1378R	TGTGCAAGGAGCAGGGAC
Fungal 18S	V4	350 bp	528F	GCGGTAATTCCAGCTCCAA
			706R	AATCCRAGAATTTCACCTCT
	V9	200 bp	1380F	CCCTGCCHTTTGTACACAC
			1510R	CCTTCYGCAGGTTCACCTAC

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	CTTGGTCATTTAGAGGAAGTAA
			ITS1-1F-R	GCTGCGTTCTTCATCGATGC

Remark: Detailed sequencing parameters can be consulted with your local sales or Novogene support.

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

Remark: Detailed analysis contents can be consulted with your local sales or Novogene support.