

16S/18S/ITS Amplicon Metagenomic Sequencing

1. Sample Requirements

Service	Sample Type	Amount (Qubit®)	Volume	Concentration	Purity (NanoDrop™/Agarose Gel)
Amplicon-based metagenomics*	Total DNA	≥ 200 ng	≥ 40 µL	≥ 5 ng/µL	OD260/280 = 1.8-2.0; no degradation, no contamination

2. Sequencing Parameters

Platform	Illumina NovaSeq 6000
Read length	Paire-end 250bp
Recommended sequencing depth	30k/50k/100k raw tags
Data quality	Guaranteed ≥ 85% bases with Q30 or higher
Turnaround time	As fast as 16 working days, time calculation starts from the day we receive confirmation of the Sample QC report

3. Primer List of Amplicons

Novogene in-house primer pairs list (31 pairs)				
Conventional Amplicon Regions & Primers (9 pairs)				
Type	Regions	Primers	Sequence (5'→3')	Length of amplicons (bp)
Bacterial 16S	V34	341F	CCTAYGGGRBGCASCAG	450-550
		806R	GGACTACNNGGTATCTAAT	
	V4	515F	GTGCCAGCMGCCGCGGTAA	300
		806R	GGACTACHVGGGTWTCTAAT	
	V45	515F	GTGCCAGCMGCCGCGGTAA	450
		907R	CCGTCAATTCCTTTGAGTTT	
Endophytic Bacteria	V57	799F	AACMGGATTAGATACCKG	400-500
		1193R	ACGTCATCCCCACCTTCC	
Archaeal 16S	V45 (Novel Archaea V4)	Arch519F	CAGCCGCCGCGGTAA	400-500
		Arch915R	GTGCTCCCCGCCAATTCCT	
Protist 18S	V4	528F	GCGGTAATTCAGCTCCAA	350
		706R	AATCCRAGAATTTACCTCT	
Fungal ITS	ITS1-5F	ITS5-1737F	GGAAGTAAAAGTCGTAACAAGG	200-400
		ITS2-2043R	GCTGCGTTCTTCATCGATGC	
	ITS2	ITS3-2024F	GCATCGATGAAGAACGCAGC	380
		ITS4-2409R	TCCTCCGCTTATTGATATGC	
Endophytic Fungi	ITS1-1F	ITS1F	CTTGGTCATTAGAGGAAGTAA	200-400
		ITS2	GCTGCGTTCTTCATCGATGC	

Unconventional Amplicon Regions & Primers (22 pairs)				
Type	Regions	Primers	Sequence (5'→3')	Length of amplicons (bp)
16S	V1-2	27F	AGAGTTTGATCCTGGCTCAG	300-400bp
		338R	TGCTGCCTCCCGTAGGAGT	
	V1-2	16SV12-F	AGRGTTYGATYCTGGCTCAG	310bp
		16SV12-R	GCTGCCTCCCGTAGGAGT	
	V1-3	27F	AGAGTTTGATCCTGGCTCAG	Around 500b
		544R	ATTACCGCGGCTGCTGG	
	V3	341F	CCTACGGGNGGCWGCAG	190bp
		805R	GACTACHVGGGTATCTAATCC	
	V3		ACTCCTACGGGAGGCAGCAG	190
			TTACCGCGGCTGCTGGCAC	
18S	V9	515F	GTGYCAGCMGCCGCGGTAA	400-500bp, around 450bp
		926R	CCGYCAATTYMTTTRAGTTT	
18S	V9	Euk1391f	GTACACACCGCCCGTC	260 +/- 50
		EukBr	TGATCCTTCTGCAGGTTACCTAC	
Fungi	ITS1	ITS86F	GTGAATCATCGAATCTTTGAA	300-400bp, around 360bp
		ITS4 (R)	TCCTCCGCTTATTGATATGC	
Arbuscular mycorrhizal fungi		AMV4-5NF	AAGCTCGTAGTTGAATTCG	200-300bp
		AMDGR	CCCAACTATCCCTATTAATCAT	
Archaea		515FmodF	GTGYCAGCMGCCGCGGTAA	200-400bp, around 300bp
		806RmodR	GGACTACNVGGGTWTCTAAT	
18S	V4-5	Eukv4F	CCAGCASCYGCGTAATTC	410bp
		Eukv5R	ACTTTCGTTCTTGATYRA	
		1380F	CCCTGCCHTTTGTACACAC	200
		1510R	CCTTCYGCAGGTTACCTAC	
		573F	CGCGGTAATTCCAGCTCCA	380bp
		951R	TTGGYRAATGCTTTCGC	
18S (metazoa)		Uni18S	AGGGCAAKYCTGGTGCCAGC	310-620bp
		Uni18SR	GRCGGTATCTRATCGYCTT	
Cyanobacteria (cyanobacteria)		CYA359F	GGGGAATYTTCCGCAATGGG	422bp
		CYA781R	GACTACWGGGGTATCTAATCCCWTT	
Archaea	1106F	1106F	TTWAGTCAGGCAACGAGC	315
		1837R	TGTGCAAGGAGCAGGGAC	
Functional gene	nifH	PolyF	TGCGAYCCSAARGCBGACTC	360
		PolyR	ATSGCCATCATYTCRCCGGA	
		nifHF	AAAGGYGGWATCGGYAARTCCACCAC	450bp
		nifHR	TTGTTSGCSGCRATCATSGCCATCAT	
	Denitrifying bacteria	cd3aF	GTSAACGTSAAGGARACSGG	426bp

	nirS	R3cdR	GASTTCGGRTGSGTCTTGA	
Mitochondria	COI gene	mICOIntF	GGWACWGGWTGAACWGTWTAYCCYCC	
		jgHCO2198	TAIACYTCIGGRTGICCAARAAYCA	
Chloroplast	TrnL	F-TrnL(UAA)c	CGAAATCGGTAGACGCTACG	150-210bp
		F-TrnL(UAA)c	CCATTGAGTCTCTGCACCTATC	
	Ammonia-oxidizing archaea (amoA- AOA)	Arch-amoA26F	GACTACATMTTCTAYACWGAYTGGGC	415bp
		Arch- amoA417R	GGKGTCA TRTATGGWGGY AAYGTTGG	

4. Data Analysis Content

Amplicon-based metagenomics sequencing (Qiime1)

Standard Analysis	Software
Data split and reads merging	Cutadapt, FLASH
Data quality control: data filtration and chimera removal	Fastp, Vsearch
Taxonomic Annotation and Analysis: Clustering-OTUs (Operational Taxonomic Units), phylogenetic tree, Taxonomy annotation (16S/18S: Silva, ITS: Unite), Relative abundance bar graph, Cluster heatmap, Ternary plot, Evolutionary tree, Venn and Flower diagram	Uparse, Qiime, muscle, R, perl
Alpha-diversity Analysis: Alpha Indices table (Observed species, Good coverage, Chao1, ACE, Shannon, Simpson, PD whole tree), Rarefaction curves, Species accumulation box plot, Rank abundance curves Alpha diversity indices differences box plot.	QIIME, R
Beta-diversity Analysis: Beta diversity heatmap, UPGMA (Unweighted Pair-group Method with Arithmetic Means), PCA (Principal Component Analysis), PCoA (Principal Co-ordinates Analysis), NMDS (Non-Metric Multidimensional Scaling)	QIIME, R, perl
Community Differences Analysis: Anosim, MRPP, Adonis, Simper, Species T-test analysis, Metagenomeseq, LEfSe analysis	R, LEfSe

Advanced Analysis	Software
Spearman, CCA/RDA, VPA analysis	R
Network analysis	R
Function prediction analysis: PICRUST2, PICRUST, Tax4Fun, FAPROTAX, FunGuild, BugBase	picrust2, picrust, tax4fun, python, R

Amplicon-based metagenomics sequencing (Qiime2)

Standard Analysis	Software
Data split and reads merging	Cutadapt, FLASH
Data quality control: data filtration and chimera removal	Fastp, Vsearch
Taxonomic Annotation and Analysis: denoise-ASVs (DADA2/deblur, default:DADA2), Taxonomy annotation (16S/18S: Silva, ITS: Unite), Relative abundance bar graph, Cluster heatmap, Ternary plot, Evolutionary tree, Venn and Flower diagram	QIIME2
Alpha Diversity Analysis: Alpha Indices table (Observed species, Good coverage, Chao1, ACE, Shannon, Simpson, PD whole tree), Rarefaction curves, Species accumulation box plot, Rank abundance curves Alpha diversity indices differences box plot.	QIIME2, R
Beta-diversity Analysis: Beta diversity heatmap, UPGMA (Unweighted Pair-group Method with Arithmetic Means), PCA (Principal Component Analysis), PCoA (Principal Co-ordinates Analysis), NMDS (Non-Metric Multidimensional Scaling)	QIIME2, R, perl
Community Differences Analysis: Anosim, MRPP, Adonis, Simper, Species T-test analysis, Metagenomeseq, LEfSe analysis	R, LEfSe
Function Prediction Analysis, (Select one) PICRUST2, PICRUST, Tax4Fun, FAPROTAX, FunGuild, BugBase	picrust2, picrust, tax4fun, python, R

Advanced Analysis	Software
Spearman, CCA/RDA, VPA analysis	R
Network analysis	R
Function prediction analysis: PICRUST2, PICRUST, Tax4Fun, FAPROTAX, FunGuild, BugBase	picrust2, picrust, tax4fun, python, R