

II. Starting Material Provided by Client

- Valid Purchase Order (PO) number or charging instructions;
- Signed quotation;
- Sample submission form, including index sequences for de-multiplexing;
- Genomic DNA samples, ≥ 0.05 ng/ μ L, $\geq 15\mu$ L, clearly labeled and numbered, submitted on blue ice or dry ice.

III. Service Description

16S sequencing of genomic DNA samples. Samples will be checked for quality upon receipt. Service includes initial sample QC, MetaVx™ library preparation, sequencing, data analysis, and data delivery. Sequencing will be performed on the Illumina PE250 platform, in a 2x250bp paired-end (PE) configuration, with at least 50K reads of data per sample. AllBio will generate data that meets or exceeds quality standards set by the system manufacturer.

Notes:

1. For Illumina sequencing, AllBio guarantees an average Q score of greater than Q30 with 70% of sequencing data.
2. Sequencing recommendations provided by AllBio are based on the Client's project goals, typical machine outputs and multiplexing variability. AllBio does not guarantee average coverage or on-target specificity for each sample.
3. Multiplexing is performed to the best of AllBio's ability to ensure relatively even data distribution amongst samples, but even data distribution is not guaranteed.
4. The sequencing results will be accessible at your AllBio Cloud account for 20 days and stored in our offline server for the next two months after delivery of the final report, free of charge. Resending the data will incur a fee for hard disks and delivery. On request of data copying after 2 months, an additional charge (30 USD/time) will be applied if the data is still available.
5. Libraries prepared by AllBio are not guaranteed and will be performed to the best of AllBio's ability. Results will depend on the bacterial/archaeal load present in each sample.
6. AllBio will store the customer samples up to 2 months since the project is delivered. The samples will be disposed after that. AllBio will not return samples, we suggest that you could keep a part of samples as backups before shipping them out.
7. For research use only, not intended for use in a clinical investigation or diagnosis.

IV. Data analysis

Bioinformatics analysis for microorganism diversity (16S)

Microflora Diversity	Bioinformation Analysis	Range of Application	Yes/No
Standard analysis	1. Data processing		
	1.1 data statistics		Yes
	1.2 quality optimization		Yes
	2. OUT(ASV) Analysis and Species annotation		
	2.1 OTU clustering, and OTU statistics		Yes
	2.2 OTU heatmap diagram	Number of samples ≥ 2	Yes

	2.3 OTU Venn or flower diagram	Venn diagram: Number of samples /groups ≥ 2 & ≤ 5 ; Flower diagram: Number of samples /groups > 5 ;	Yes
	2.4 Species annotation		Yes
	2.5 Graphics display	Only for: Top30 species	Yes
	2.6 Phylogenetic Tree of the Genus	Only for: Top30 species	Yes
	2.7 Krona species composition diagram		Yes
	3.Alpha Diversity		
	3.1 Alpha diversity Analysis		Yes
	3.2 group differential Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	Yes
	3.3 Rank-Abundance curve		Yes
	3.4 Rarefaction curve		Yes
	3.5 Pan/Core Curve	Number of samples ≥ 5	Yes
	3.6 Species Accumulation Curve	Number of samples ≥ 5	Yes
	4.Beta Diversity		
	4.1 (Un) weighted unifrac unifrac distance matrix	Number of samples ≥ 3	Yes
	4.2 PCoA Analysis	Number of samples ≥ 3	Yes
	4.3 PCA Analysis	Number of samples ≥ 3	Yes
	4.4 NMDS Analysis	Number of samples ≥ 3	Yes
	4.5 UPGMA Tree	Number of samples ≥ 3	Yes
	4.6 Anosim Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	Yes
	4.7 Adonis Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	Yes
	5.Differential Analysis of community composition		
	5.1 Wilcoxon Rank-Sum test Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	Yes
	5.2 LEfse Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	Yes
	5.3 STAMP Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	Yes
Advanced Analysis	5.4 MetagenomeSeq Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	NO
	5.5 GraPhlAn Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	NO
	6. Functional Predictive Analysis		
	6.1 PICRUST2 Prediction Analysis	Number of samples ≥ 5	NO
	6.2 Metabolic Pathway Differential Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	NO
	6.3 BugBase Phenotype Prediction	Number of samples ≥ 5	NO
	6.4 Tax4Fun Prediction Analysis	Number of samples ≥ 5	NO
	6.5 FAPROTAX Prediction Analysis	Number of samples ≥ 5	NO

6.6 FunGuild Prediction Analysis	Number of samples ≥ 5 , Only for fungi	NO
7. Environmental Factor Correlation Analysis		
7.1 Environmental Factor Correlation Heatmap	Number of samples ≥ 3 , Number of environmental factors ≥ 2	NO
7.2 RDA/CCA Analysis	Number of samples ≥ 3 , Number of environmental factors < Number of samples	NO
7.3 VIF Analysis	Number of samples ≥ 3 , Number of environmental factors ≥ 2	NO
7.4 Mantel test Analysis	Number of samples ≥ 3 , Number of environmental factors < Number of samples	NO
7.5 VPA Analysis	Number of samples ≥ 3 , Number of environmental factors < Number of samples	NO
7.6 Procrustes Analysis	Number of samples ≥ 3 , Number of environmental factors < Number of samples	NO
8. Model Validation and Network Analysis		
8.1 RandomForest Analysis	Number of samples ≥ 5	NO
8.2 ROC Analysis	Number of groups = 3, Number of samples per group ≥ 3	NO
8.3 PLS-DA Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	NO
8.4 Enterotype Analysis	Number of groups ≥ 2 , Number of samples per group ≥ 3	NO
8.5 Phylogenetic Evolutionary Tree		NO
8.6 Collinearity Analysis		NO
8.7 Network Analysis	Number of samples ≥ 5	NO
9. Multi-Omics Analysis (The following analysis requires metabolome data)		
9.1 Multi-omics correlation heatmap	Number of samples ≥ 3 , Number of metabolites ≥ 2	NO
9.2 Plot		NO
9.3 Redundancy analysis	Number of samples ≥ 3 , Number of metabolites < Number of samples	NO
9.4 Associated Network Diagram		NO
10. Personalized Analysis		

Notes:

1. Before analysis, client needs to provide sample name, grouping information and order (effect the sample order in figure)
2. If sample name appears symbols that do not belong to number or alphabet, which will be removed when analyze.
3. Advanced analysis (individuation analysis) needs to extra charges assessed.
4. All figures in the analytical content show form reference to template report, if want to add or modification figure, needs to extra charges assessed.