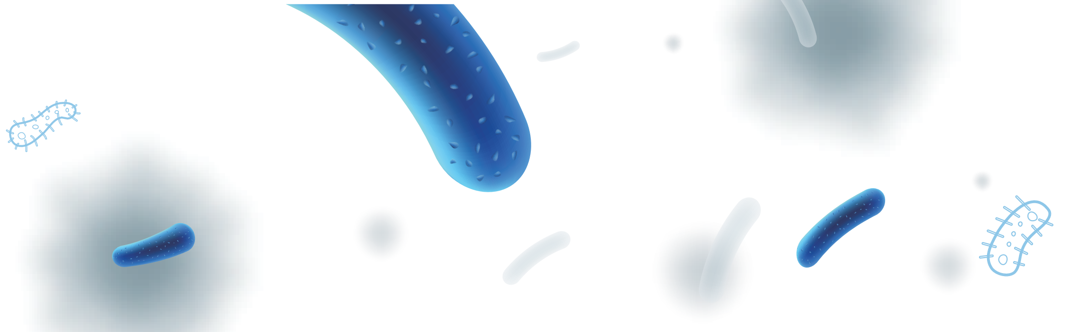


MGI 16S rDNA Sequencing Products Package

Fast, accurate, and easy to use, providing a powerful tool for microbial community research

Features

- Designed to cover the V3-V4 regions, which can provide genera-to-species level taxonomic resolution.
- Simple library preparation with ATOPlex 2-step PCR method within 6 hours.
- DNBSEQ-G99 sequencing completes PE300 sequencing within 30 hours, providing a rapid turnaround time for your research.
- Analysis software includes various functions, such as OTU analysis, taxonomic level identification, alpha diversity analysis, beta diversity analysis, PCA/PCoA, and cluster/heatmap analysis.
- Up to 4608 unique barcodes are available for library preparation, meets high throughput sequencing requirements.

Introduction

Microorganisms are ubiquitous in various environments such as the ocean, soil, homes, hospitals, and the human body, and they play a crucial role in different ecosystems. Analyzing the diversity of microorganisms in different microbial communities is essential to gain a comprehensive understanding of life and has significant implications for human health, environmental science, and industry.

The DNBSEQ-G99 is among the fastest small-to-medium-throughput sequencers in the world, capable of performing PE300 sequencing in just 30 hours while delivering high-quality data output. The DNBSEQ-G99 is also available with an integrated bioinformatics module, enabling automated data analysis on-machine.

The ATOPlex 16S V3V4 Library Prep Set is developed based on the ATOPlex Library Preparation technology, and covers the variable regions V3-V4 of the 16S ribosomal RNA gene. This method utilizes a 2-step PCR protocol to first amplify the targeted 16S V3-V4 region, followed by a second PCR step to add MGI sequencing adapters.

The HGT 16S/18S/ITS Metagenomics Software includes several functional modules for 16S analysis, such as OTU analysis, taxonomic level identification, alpha diversity analysis, beta diversity analysis, PCA/PCoA, and cluster/heatmap analysis.

MGI's 16S rDNA sequencing products package includes MGI's reagents, sequencer, and data analysis software, covering the entire process from sample to report. This package can be applied to research on microbial community structure, evolutionary relationships, and human health.

Table 1. Product parameters

Application	Sequencing Read length	Recommended number of reads per sample
16S V3-V4 sequencing	PE300	⩾0.1 M Reads

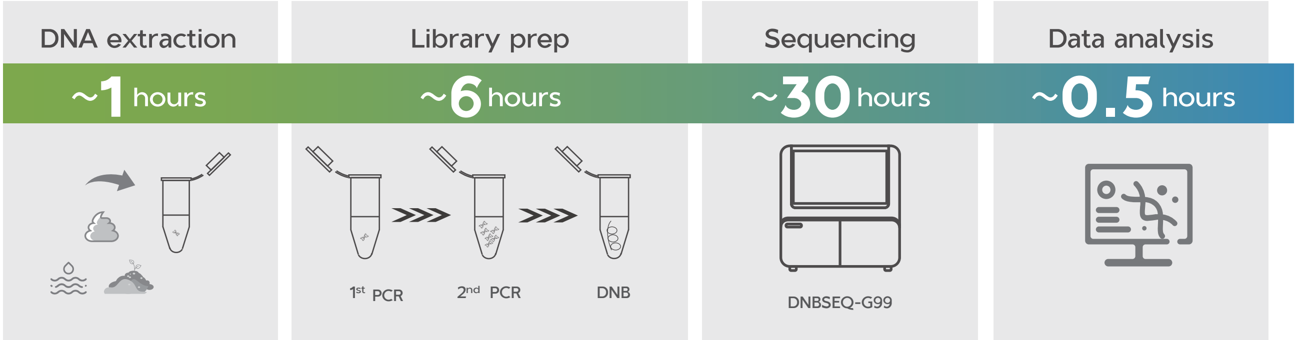
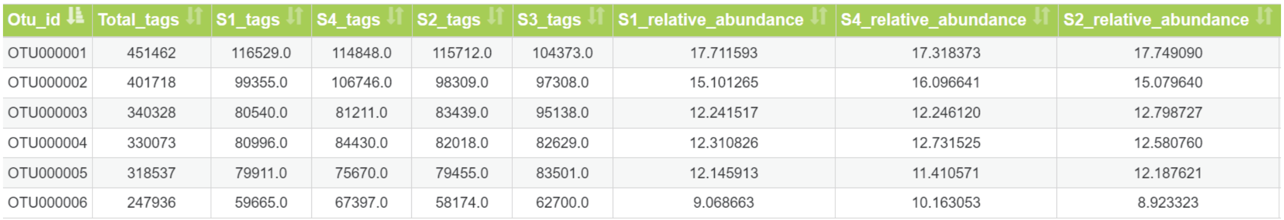


Fig 1. Workflow

Performance

Four samples were tested using the MGI 16S rDNA sequencing workflow, the software performed OTUs analysis and clustering, and calculated the abundance of each type of OTUs. The results are shown in table 2 (note that only partial results displayed).

Table 2. OTU abundance statistics



Otu_id	Total_tags	S1_tags	S4_tags	S2_tags	S3_tags	S1_relative_abundance	S4_relative_abundance	S2_relative_abundance
OTU000001	451462	116529.0	114848.0	115712.0	104373.0	17.711593	17.318373	17.749090
OTU000002	401718	99355.0	106746.0	98309.0	97308.0	15.101265	16.096641	15.079640
OTU000003	340328	80540.0	81211.0	83439.0	95138.0	12.241517	12.246120	12.798727
OTU000004	330073	80996.0	84430.0	82018.0	82629.0	12.310826	12.731525	12.580760
OTU000005	318537	79911.0	75670.0	79455.0	83501.0	12.145913	11.410571	12.187621
OTU000006	247936	59665.0	67397.0	58174.0	62700.0	9.068663	10.163053	8.923323

Subsequently, the number of tag sequences for each sample at each classification level (kingdom, phylum, class, order, family, genus, species) was counted based on the species annotation information of OTUs. The results were used to generate histograms and Krona diagrams.

Table 3. Number of tags assigned to different taxonomic levels

	Domain	Phylum	Class	Order	Family	Genus	Species
S1	657901	657872	657872	657205	656320	653759	494244
S2	651913	651892	651892	651204	650342	647811	492473
S3	660263	660228	660228	659658	658844	656497	503129
S4	663126	663097	663097	662454	661685	659223	498926

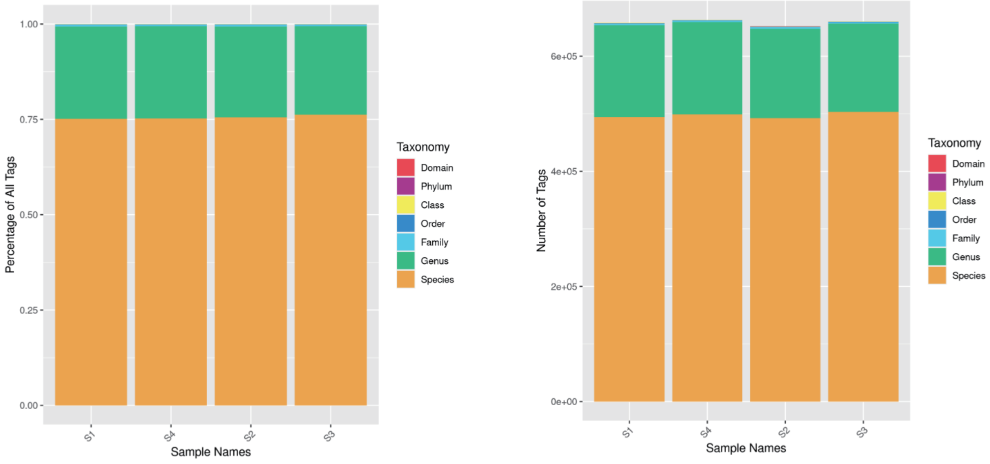
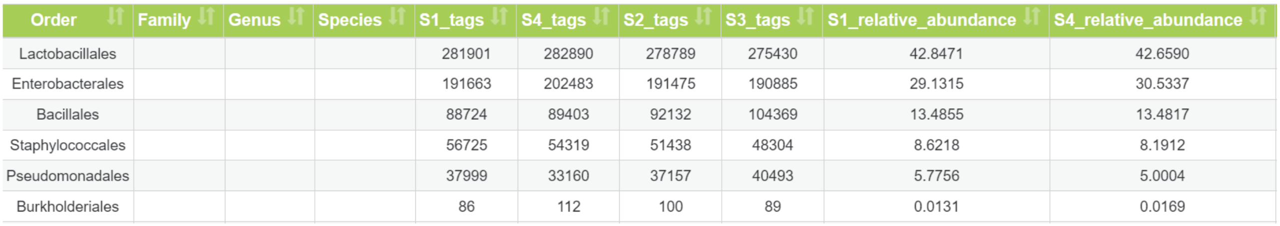


Fig 2. Histogram of the percentage of each sample assigned to each classification level

Fig 3. Krona diagram

Table 4. Number of tags associated with different orders for various samples



Order	Family	Genus	Species	S1_tags	S4_tags	S2_tags	S3_tags	S1_relative_abundance	S4_relative_abundance
Lactobacillales				281901	282890	278789	275430	42.8471	42.6590
Enterobacterales				191663	202483	191475	190885	29.1315	30.5337
Bacillales				88724	89403	92132	104369	13.4855	13.4817
Staphylococcales				56725	54319	51438	48304	8.6218	8.1912
Pseudomonadales				37999	33160	37157	40493	5.7756	5.0004
Burkholderiales				86	112	100	89	0.0131	0.0169

Microbial communities are typically comprised of a few dominant species and a few rare species, with the dominant species playing a significant role in determining the function of the community. The software determines the taxonomic composition at each classification level for each sample and generates a stacked bar chart for visualizing differences in species abundance across different samples. The top 10 species, with relative abundances greater than or equal to 2% in at least one sample, are displayed in the chart, with all other species being classified as 'Other'. OTUs which are not annotated at the stipulated classification level will be indicated as 'unclassified'.

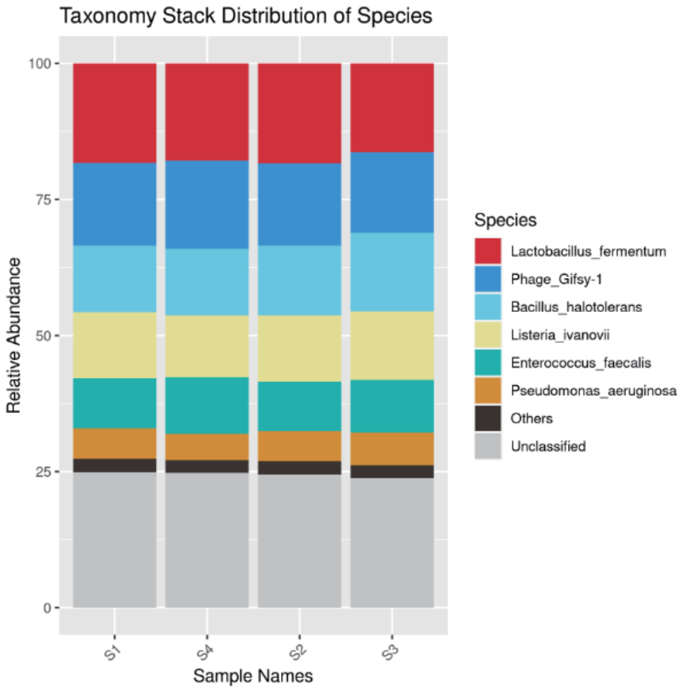


Fig 4. Stacked bar chart of species distribution for various samples

The following cluster heatmap displays the abundance of different species in each sample. Clustering relationships reflected on the heat map can facilitate understanding of hierarchical clusters within samples.

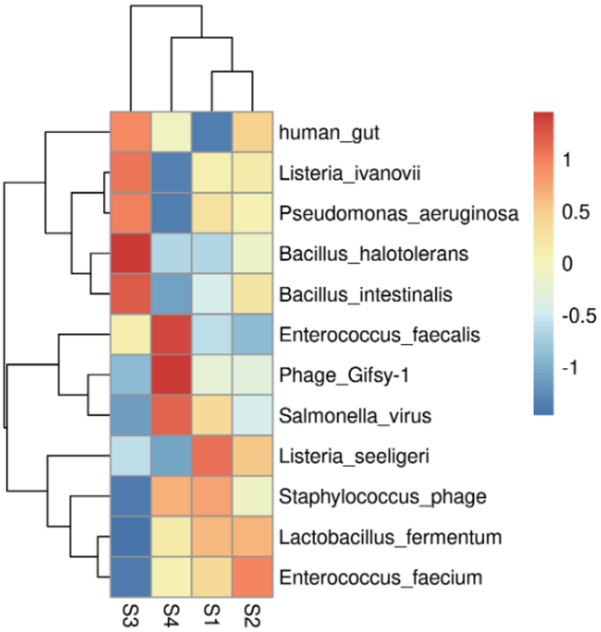


Fig 5. Cluster heatmap of species abundance for various samples

Alpha diversity indicators provide information on the diversity (reflects number of taxonomic groups) and evenness (reflects distribution of group abundance) of microbial communities in the sample. Results are as shown in the following figures.

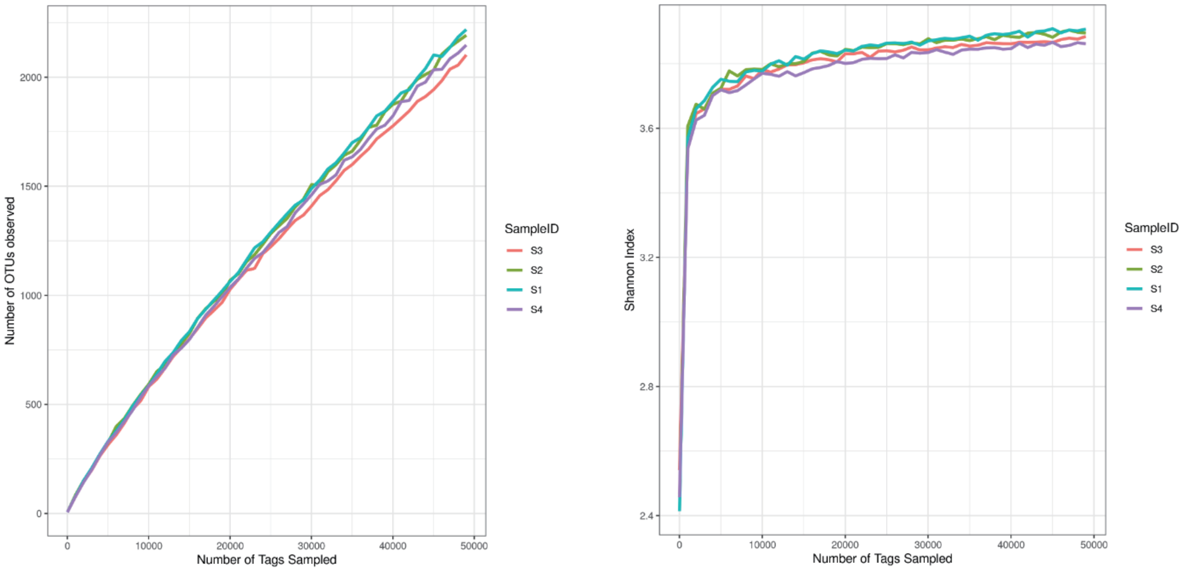


Fig 7. Shannon Rarefaction Curve

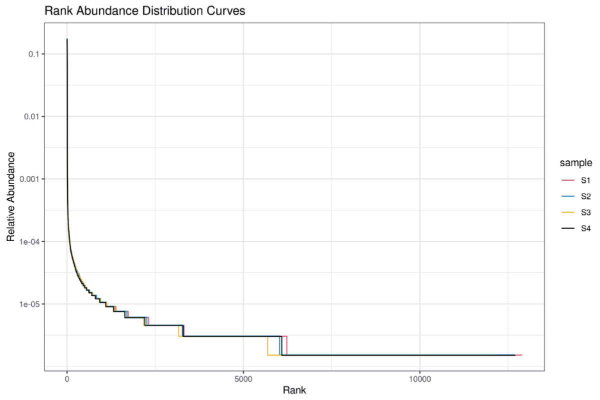


Fig 8. Rank abundance analysis of the different bacterial community groups

The software utilizes the Unifrac distance (weighted or unweighted) to measure the phylogenetic differences between samples by considering evolutionary trees estimated based on sequence similarity. The results are presented in the form of a heatmap, which is used to visualize the differences in phylogenetic composition between samples as shown below.

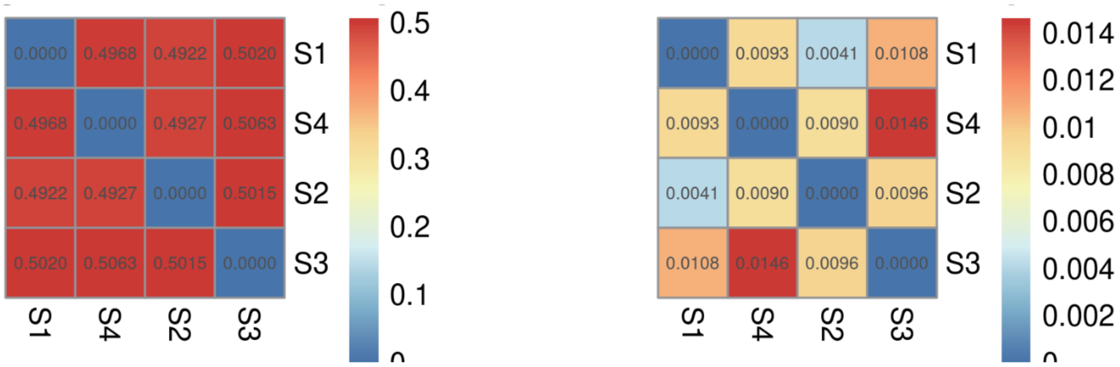


Fig 9. Heatmap of Unweighted and Weighted Unifrac indices

Principal component analysis (PCA), Principal coordinate analysis (PCoA), and Nonmetric multidimensional scaling (NMDS) analysis results are presented in Figure 10. In these graphs, samples with similar compositions are represented by points that are closer together. In theory, samples in similar environments often exhibit clustering.

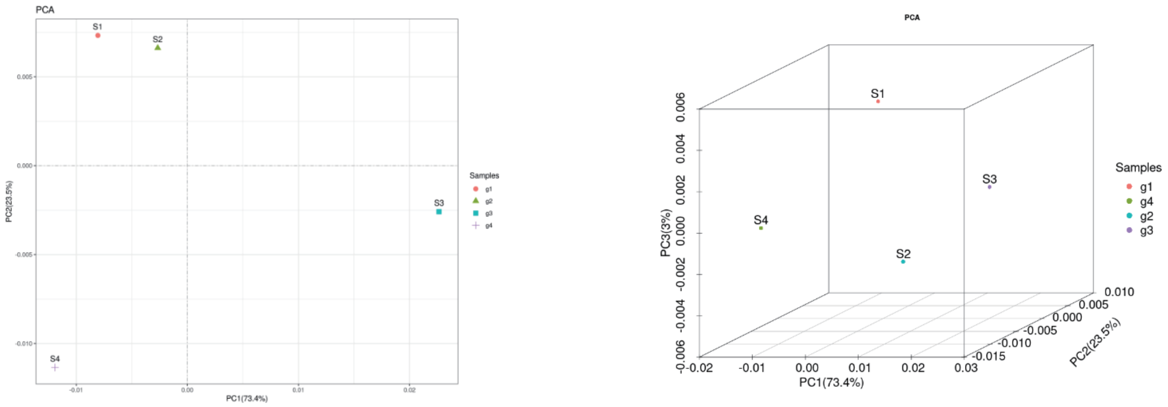


Fig 10. 2D and 3D OTU PCA plot

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis is a simple hierarchical clustering method (bottom-up) that can be used to study the similarity between samples. UPGMA analysis is based on weighted and unweighted Unifrac data and is conducted using Mothur software. The resulting UPGMA classification tree is used to visualize the similarity between samples, with more similar samples being represented by shorter common branches on the tree. The analysis results are presented in the figure below.

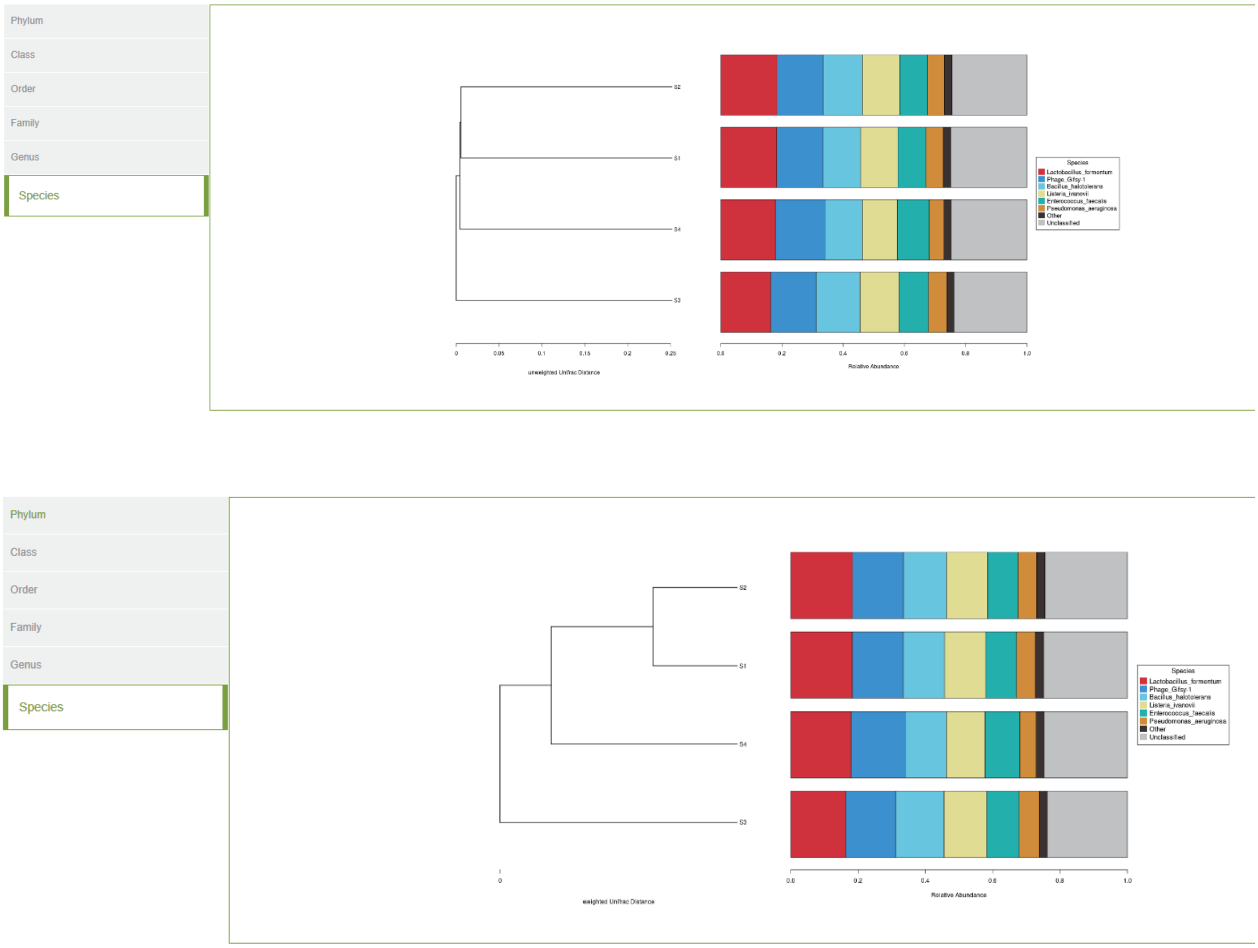


Fig 11. UPGMA Clustering Tree

Ordering information

Products	Specifications	Item No.
Sequencer		
DNBSEQ-G99ARS	Include server	900-000609-00
Reagent		
MGIEasy Stool Microbiome DNA Extraction Kit	96 RXN	940-000122-00
ATOPlex 16SV3V4 rDNA Library Preparation Set	96 RXN	940-001261-00
ATOPlex 16SV3V4 rDNA Library Preparation Set	576 RXN	940-000725-00
ATOPlex E450 Dual Barcode Balanced Library Reagent	40ng/tube	940-000637-00
MGIEasy Dual Barcode Circularization Kit V1.0	-	1000020570
DNBSEQ-G99RS High-throughput Sequencing Set	G99 SM FCL PE300	940-000415-00
Software		
HGT 16S/18S/ITS Metagenomics Software	/	970-000333-00

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