Microbiome Metabarcoding Sequencing Package

——Widely applicable and precise analysis, providing efficient tools for microbiome research

Product Features

- **Widely Application Scenarios**
  Designed based on the popular variable regions of rDNA, compatible with various sample types, fully meeting the needs of microbiome research in human, industrial, agricultural, and environmental scenarios;
- **One-stop Product Solution**
  Integrated nucleic acid extraction, library preparation, sequencing, and analysis products, meeting customer needs in one stop;
- **Superior Data Quality**
  Unique DNBSEQ sequencing technology provides high-quality sequencing data;
- **Powerful Analysis functions**
  Self-developed analysis software offers a wealth of analytical functions, such as OTU analysis, species composition analysis, Alpha diversity analysis, Beta diversity analysis, biomarker analysis, etc., and support for customized databases;
- **Supports Large-scale Parallel Sequencing**
  Up to 4608 barcodes can be customized for library preparation, meeting the needs of ultra-high throughput sequencing and saving costs to the greatest extent.

Product Introduction

Microorganisms are ubiquitous in almost all environments on Earth, such as the human body, soil, plants, and industrial environments. They play a crucial role in various ecosystems. Microbiome research aids in promoting and improving human health and is significant for ecological protection, industrial, and agricultural development. 16S/ITS rDNA sequencing provides a rapid and cost-effective method for classifying microbes at the genus-species level and is a widely used technique for microbial community analysis.

MGI's Microbiome Metabarcoding Sequencing Package is based on self-developed nucleic acid extraction reagents, ATOPlex library preparation reagents, MGISP-960 automated sample preparation systems, DNBSEQ-G99 and DNBSEQ-E25 sequencers, and MetaSIS analysis software. It covers the entire process from sample processing to analytical reporting, enabling rapid and accurate analysis of biological community composition in environmental, human, industrial, and agricultural samples, providing a tool for microbiome research.

Table 1. Product Parameters

Recommended Sample Types	Feces, oral swabs, skin swabs, tongue coating swabs, saliva, rhizosphere soil, leaf surfaces, fermented grains, yogurt, sediment, etc.
Applicable Species	Bacteria, Fungi
Recommended Sample Input	DNA:10 ng
Recommended Sequencing Read Length	16SV3V4/ITS1/ITS2:PE300; 16SV4:PE150
Recommended Data Volume	⩾65K reads per sample
Recommended Maximum Sequencing Throughput	DNBSEQ-G99 PE150:500 Samples / FC
	DNBSEQ-G99 PE300.300 Samples / FC
	DNBSEQ-E25 PE150: 72 Samples / FC
Shortest Reporting Period	20.5 hrs

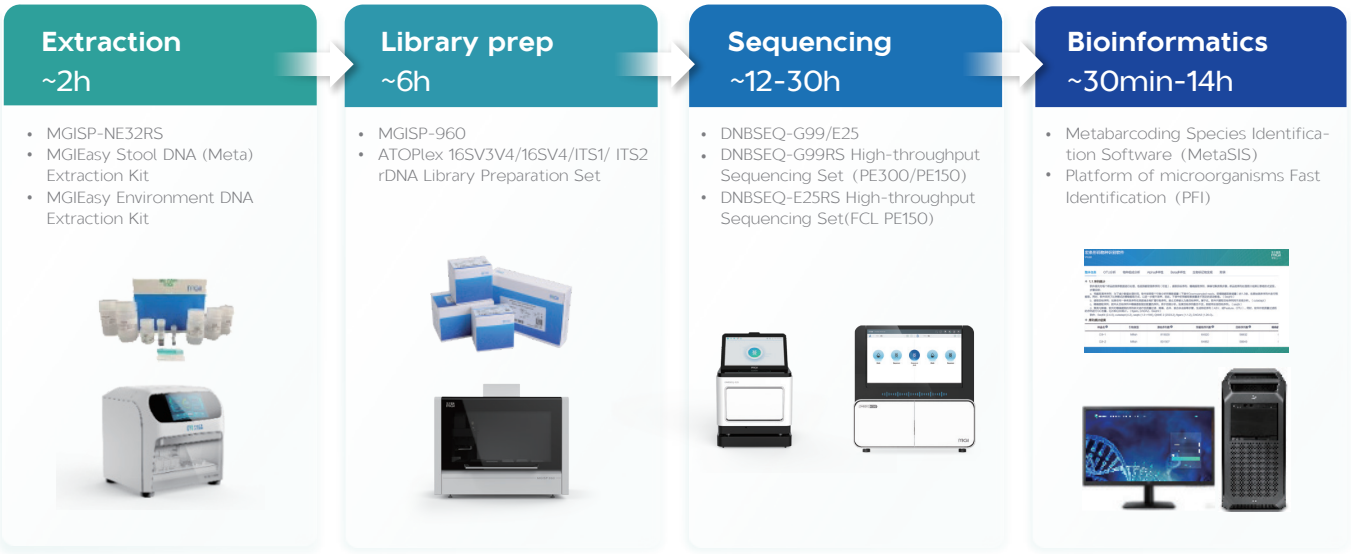

Figure 1. Workflow diagram

Product Performance

The following three sets of data were obtained from sequencing analysis of 16S V3-V4 and 16S V4 using Zymo standard to assess species detection rate, species abundance accuracy, and reproducibility. As shown in Figures 2, 3, and 4, all libraries detected the 8 expected bacterial species, with a 100% accuracy rate in species identification (at the genus level), and the difference between the bacterial abundance and the expected abundance was less than 10%. Additionally, the reproducibility between batches and within batches was good.

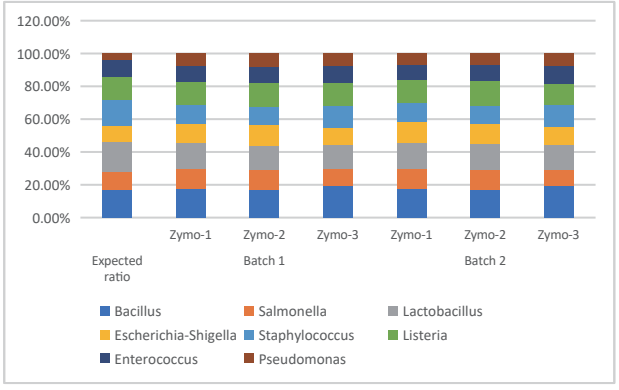

Figure 2. Stacked Bar Chart of Bacterial Abundance for Zymo Standard (DNBSEQ-G99_16SV3V4)

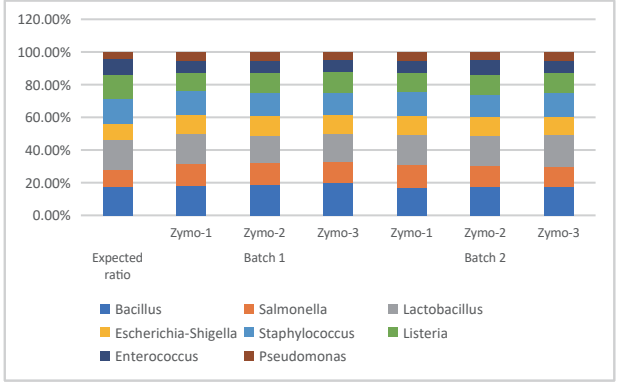

Figure 3. Stacked Bar Chart of Bacterial Abundance for Zymo Standard (DNBSEQ-G99_16SV4)

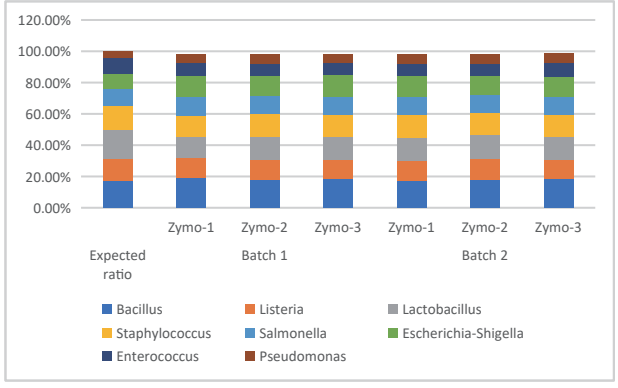

Figure 4. Stacked Bar Chart of Bacterial Abundance for Zymo Standard (DNBSEQ-E25_16SV4)

Microorganisms form different ecological communities in many parts of the human body, such as the gut, mouth, and skin, and play an important role in maintaining health. The following three sets of data are the results of 16S V3-V4, 16S V4, ITS1, and ITS2 sequencing analyses performed on human fecal, oral swab, and skin swab samples. As shown in Figures 5 and 6, two fecal DNA samples were analyzed by 16S V3-V4 and 16S V4 sequencing. One sample was identified to contain Prevotella, Alloprevotella, and *Lachnospira*, with *Prevotella* being the dominant genus in the sample. Another sample was identified to contain Bacteroides, *Phascolarctobacterium*, and *Parabacteroides*, with Bacteroides being the dominant genus, all of which are important bacterial in the human gut. As shown in Figures 7 and 8, one fecal DNA sample was analyzed by ITS1 and ITS2 sequencing, identifying *Saccharomyces*, *Candida*, and *Nigrospora*, all of which are common fungal in the gut.

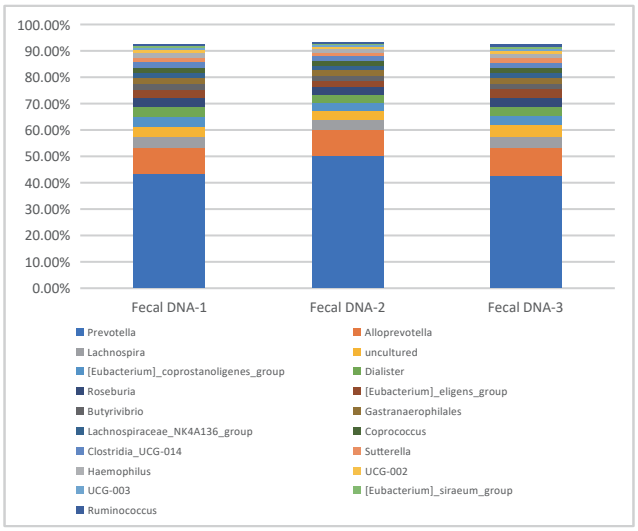

Figure 5. Stacked Bar Chart of Bacterial Abundance for fecal sample (DNBSEQ-G99_16SV3V4)

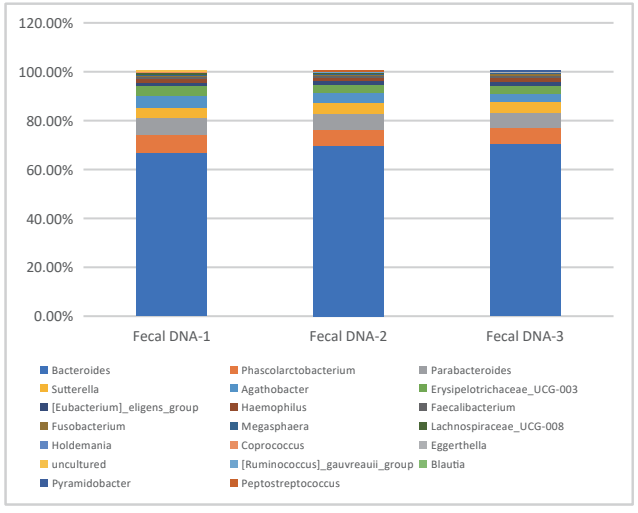

Figure 6. Stacked Bar Chart of Bacterial Abundance for fecal sample (DNBSEQ-E25_16SV4)

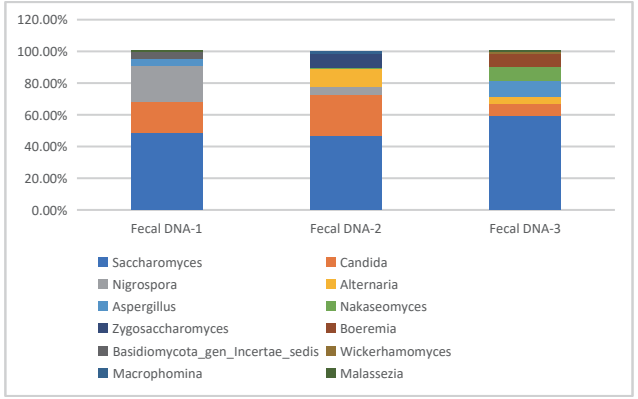

Figure 7. Stacked Bar Chart of Bacterial Abundance for fecal sample (DNBSEQ-G99_ITS1)

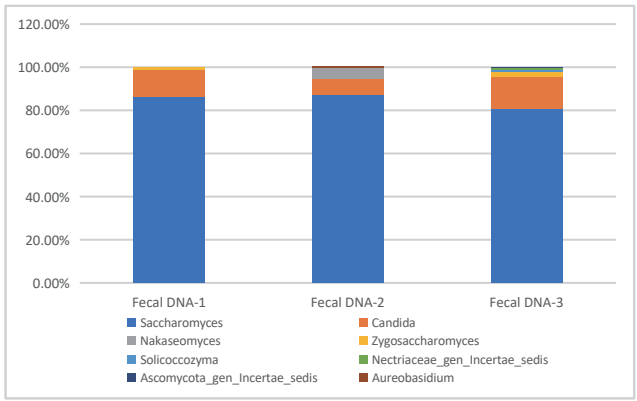

Figure 8. Stacked Bar Chart of Bacterial Abundance for fecal sample (DNBSEQ-G99_ITS2)

As shown in Figures 9 and 10, one oral swab sample was analyzed by 16S V3-V4 and 16S V4 sequencing, identifying Prevotella, *Streptococcus*, *Neisseria*, *Haemophilus*, and *Alloprevotella*, which are common bacterial in the human oral cavity, with *Prevotella* being the dominant community in this sample. As shown in Figure 11, another sample analyzed by ITS1 sequencing identified *Aspergillus* and *Candida*, which are common fungal communities in the oral cavity.

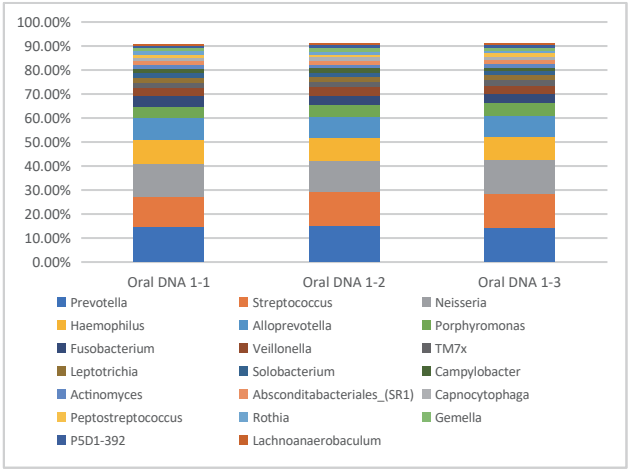

Figure 9. Stacked Bar Chart of Bacterial Abundance for oral swab (DNBSEQ-G99_16SV3V4)

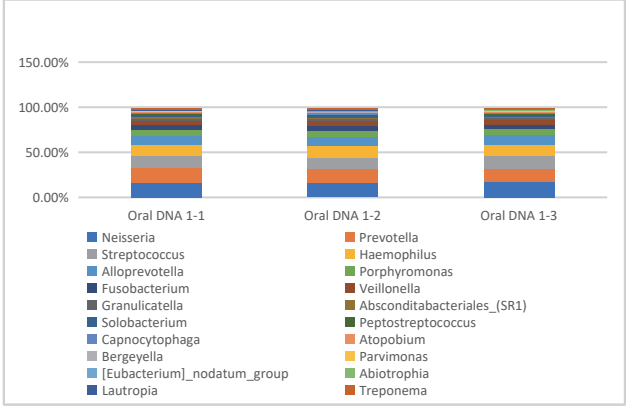

Figure 10. Stacked Bar Chart of Bacterial Abundance for oral swab (DNBSEQ-E25_16SV4)

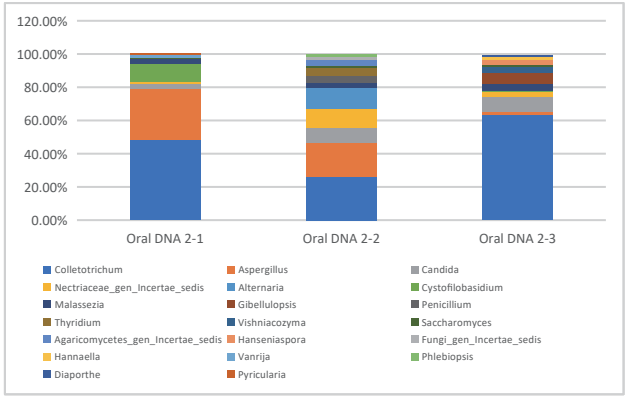

Figure 11. Stacked Bar Chart of Bacterial Abundance for oral swab (DNBSEQ-G99_ITS1)

As shown in Figures 12 and 13, two skin samples were analyzed by 16S V3-V4 and 16S V4 sequencing, respectively identifying *Staphylococcus*, *Lawsonella*, *Acinetobacter*, and *Corynebacterium*, which are common bacterial on the human skin surface. As shown in Figures 14 and 15, another two skin samples were analyzed by ITS1 and ITS2 sequencing, respectively identifying *Malassezia*, *Aspergillus*, *Epicoccum*, and *Malassezia*, *Candida*, *Alternaria*, which are common fungal on the skin surface.

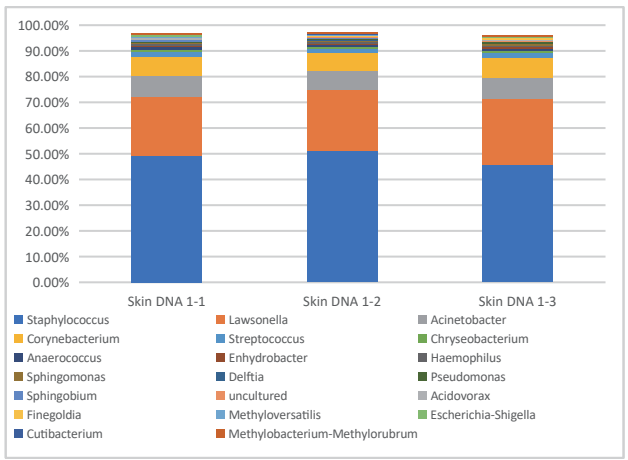

Figure 12. Stacked Bar Chart of Bacterial Abundance for skin sample (DNBSEQ-G99_16SV3V4)

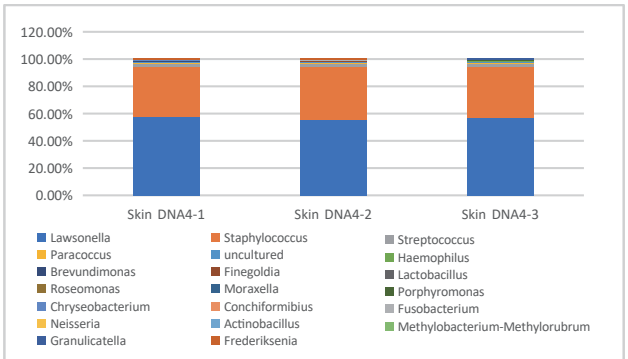

Figure 13. Stacked Bar Chart of Bacterial Abundance for skin sample (DNBSEQ-E25_16SV4)

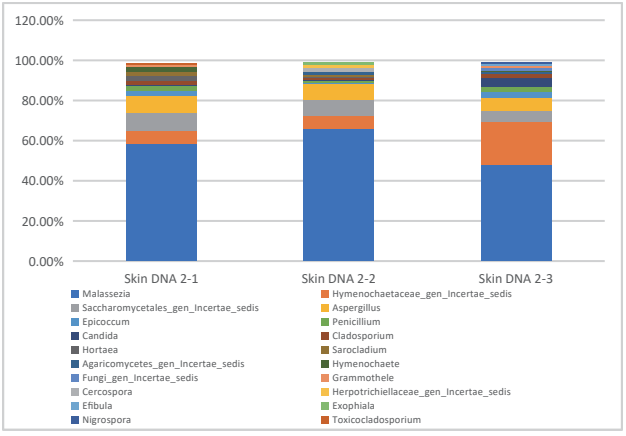

Figure 14. Stacked Bar Chart of Bacterial Abundance for skin sample (DNBSEQ-G99_ITS1)

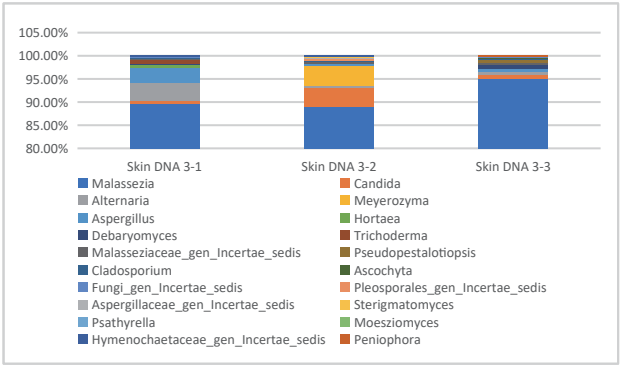

Figure 15. Stacked Bar Chart of Bacterial Abundance for skin sample (DNBSEQ-G99_ITS2)

In the field of food industry, microorganisms hold significant production value and play crucial roles in enhancing food quality, ensuring food safety, and optimizing food processing procedures. The following two sets of data are the results of 16S V3-V4, 16S V4, ITS1, and ITS2 sequencing analyses conducted on samples of fermented grain and yogurt. As depicted in Figures 16, 17, 18, and 19, one sample of fermented grain was sequenced using 16S V3-V4, 16S V4, ITS1, and ITS2, identifying common bacterial communities in fermented grain mash such as *Weissella*, *Lactobacillus*, *Oceanobacillus*, and *Pediococcus*, with Weissella being the predominant genus in this sample. Additionally, common fungal communities in fermented grain mash were identified, including *Mucor*, *Paecilomyces*, and *Aspergillus*.

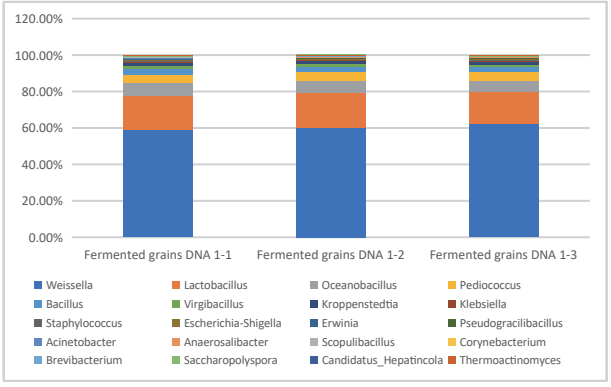

Figure 16. Stacked Bar Chart of Bacterial Abundance for fermented grain (DNBSEQ-G99_16SV3V4)

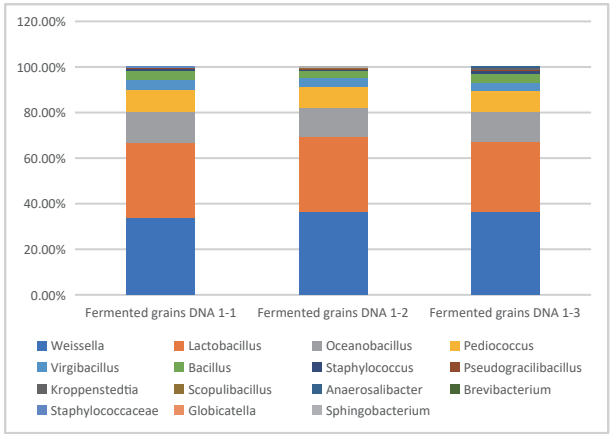

Figure 17. Stacked Bar Chart of Bacterial Abundance for fermented grain (DNBSEQ-E25_16SV4)

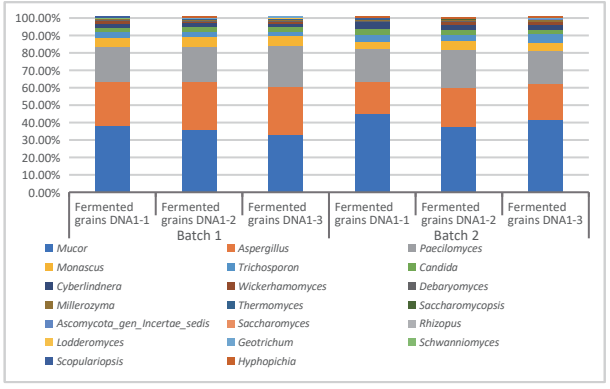

Figure 18. Stacked Bar Chart of Bacterial Abundance for fermented grain (DNBSEQ-G99_ITS1)

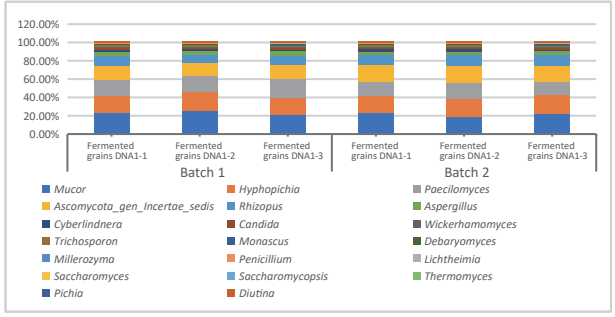

Figure 19. Stacked Bar Chart of Bacterial Abundance for fermented grain (DNBSEQ-G99_ITS2)

As shown in Figures 20 and 21, one yogurt sample was analyzed by 16S V3-V4 and 16S V4 sequencing, and both identified *Streptococcus* and *Lactobacillus*, which are the most common bacterial found in yogurt.

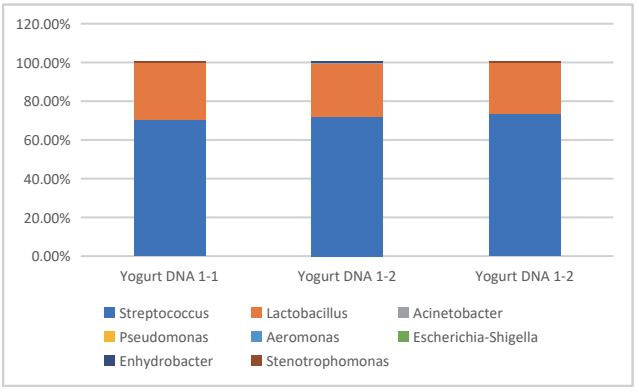

Figure 20. Stacked Bar Chart of Bacterial Abundance for yogurt sample (DNBSEQ-G99_16SV3V4)

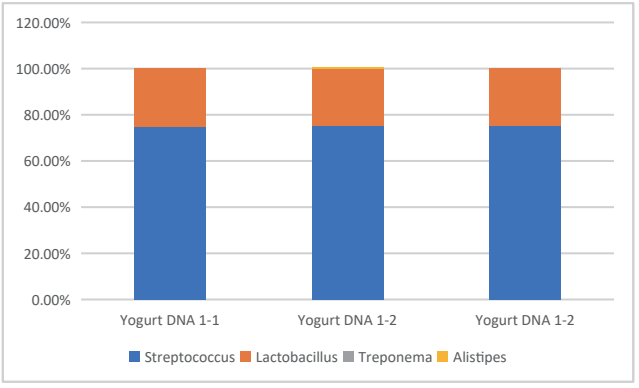

Figure 21. Stacked Bar Chart of Bacterial Abundance for yogurt sample (DNBSEQ-E25_16SV4)

Microbiomes can influence the growth and development of crops, enhance their stress resistance, and improve the quality of agricultural products through various means on the surface of crop leaves, making them of significant research value in the field of agriculture. The following data set involves two samples of leaf surface wash liquids that were analyzed using 16S V3-V4 and 16S V4 sequencing. The analysis identified *Candidatus_Portiera*, a primary endosymbiont, *Rickettsia*, *Candidatus_Hamiltonella*, a secondary endosymbiont, as well as *Pantoea*, *Pseudomonas*, and *Paenibacillus*, which are common bacterial on leaf surfaces, as shown in Figures 22 and 23. Additionally, two other samples of leaf surface wash liquids were analyzed using ITS1 and ITS2 sequencing, identifying *Cladosporium*, *Nigrospora*, *Curvularia*, *Elsinoe*, and *Lachnum*, which are common fungal that often cause leaf diseases, as shown in Figures 24 and 25.

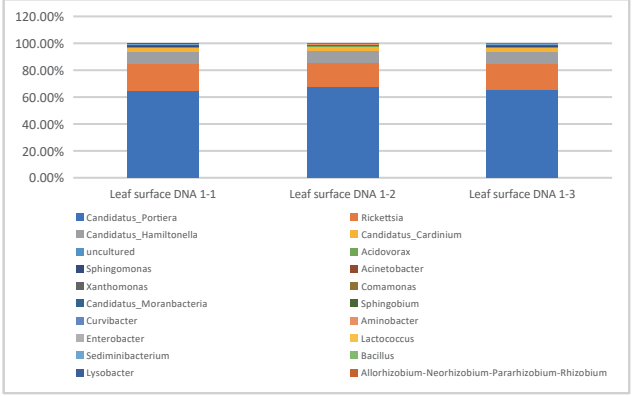

Figure 22. Stacked Bar Chart of Bacterial Abundance for leaf surface (DNBSEQ-G99_16SV3V4)

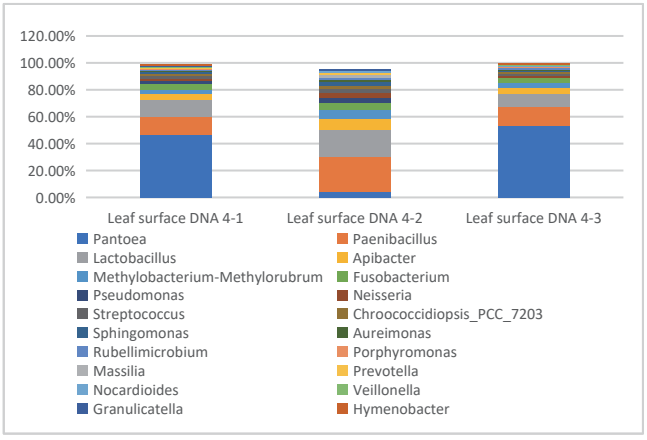

Figure 23. Stacked Bar Chart of Bacterial Abundance for leaf surface (DNBSEQ-E25_16SV4)

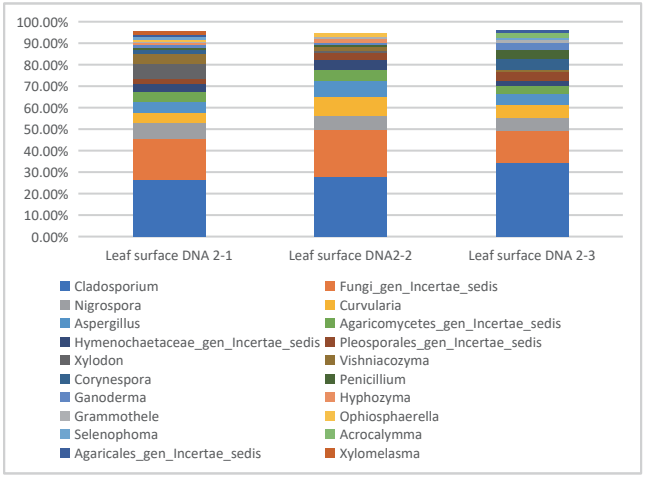

Figure 24. Stacked Bar Chart of Bacterial Abundance for leaf surface (DNBSEQ-G99_ITS1)

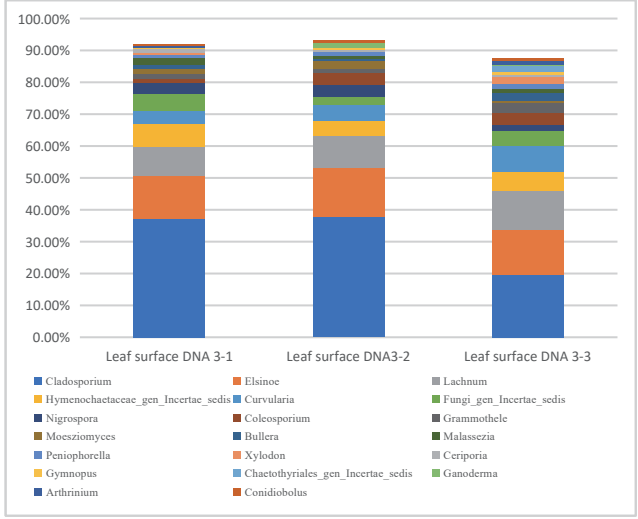

Figure 25. Stacked Bar Chart of Bacterial Abundance for leaf surface (DNBSEQ-G99_ITS2)

As land resources become increasingly scarce, the deep sea, with its rich biodiversity, has become a new frontier for resource development. The following data represents the 16S V4 analysis report for one deep-sea sediment sample (with two technical replicates) and one standard sample (with two technical replicates).

An Operational Taxonomic Unit (OTU) is a uniform identifier assigned to a classification unit to facilitate analysis in phylogenetic or population genetic studies. The MetaSIS software clusters sequences with 100% similarity into a single OTU. After obtaining the OTUs, the QIIME 2 module is used to align and annotate the OTU sequences with a reference database, yielding species identification results at different taxonomic levels. By statistically analyzing the species identification of OTUs, the composition of biological communities in each sample at various taxonomic levels (consisting of domains, phyla, classes, orders, families, genera, and species) is obtained, as shown in Table 2.

Table 2. OUT Statistics table

Sample ID	Number of OTU types	Number of OTU sequence	Number of OTU types	Number of OTU sequences	Kingdom	Phylum	Class	Order	Family	Genus	Species
Sample-1	11	41065	10	41030	1	2	2	5	7	9	9
Sample-2	13	40996	11	40956	1	2	2	5	7	9	11
Sample-3	843	32742	840	32709	2	36	86	159	182	190	54
Sample-4	840	31041	837	30980	2	40	88	158	177	190	52

Without considering the abundance, based on the species identification results of all samples, a species composition visualization graph is generated using the GraPhlAn module, as shown in Figure 26. This graph can intuitively display the overall species composition at various taxonomic levels for the samples, helping users identify dominant biological groups.

Figure 26. Species Composition Chart

Table 3 displays the relative abundance of species at different taxonomic levels (phylum, class, order, family, genus, species) for the samples. Due to space limitations, this article only presents the top ten Species. It can be observed that uncultivated bacteria and archaea, such as *Chloroflexi*, *Acidobacteria*, and *Proteobacteria*, have been detected.

Table 3. Statistical table of relative abundance of species

level	Latin name	Sample-1	Sample-2	Sample-3	Sample-4
Species	*uncultured bacterium*	15.3449%	15.6973%	57.8373%	56.6946%
Species	*Lactobacillus fermentum*	22.9442%	21.9089%	0.0000%	0.0000%
Species	*Bacillus subtilis*	13.8557%	13.0481%	0.0000%	0.0000%
Species	*Klebsiella_sp.*	11.7865%	13.7098%	0.0000%	0.0000%
Species	*Escherichia coli*	12.0741%	12.4060%	0.0000%	0.0000%
Species	*uncultured archaeon*	0.0000%	0.0000%	13.0820%	14.3544%
Species	*Listeria monocytogenes*	8.6717%	8.4823%	0.0000%	0.0000%
Species	*Pseudomonas aeruginosa*	7.4994%	7.2029%	0.0000%	0.0000%
Species	*Enterococcus faecalis*	6.3295%	5.8331%	0.0000%	0.0000%

Select the top 18 species identification results in terms of abundance at the species taxonomic level for each sample, and generate a stacked bar chart. This can intuitively display the composition of biological communities at different taxonomic levels within the samples, providing a better understanding of the differences and similarities between the samples. As shown in Figure 27.

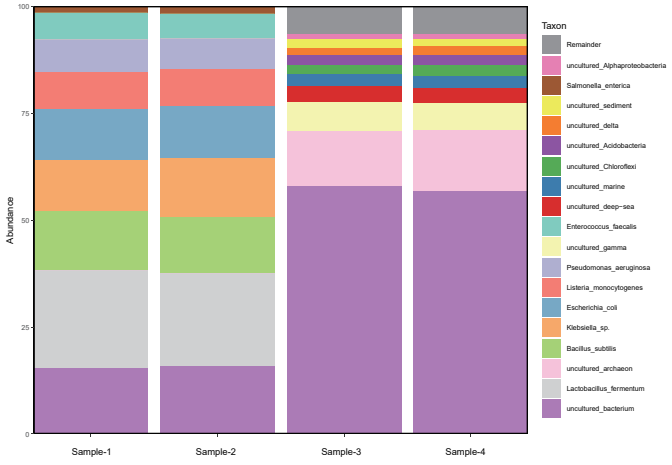

Figure 27. Species distribution stacking diagram.

Select the top 50 species identification results in terms of abundance at the species taxonomic level for each sample and generate a heatmap. This visual representation can intuitively display the composition of biological communities at different taxonomic levels within the samples, allowing for a better understanding of the differences and similarities between the samples. As shown in Figure 28.

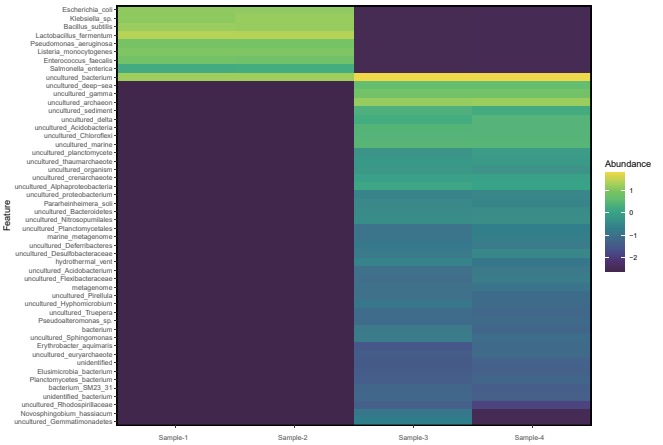

Figure 28. Species distribution heat map.

In order to more intuitively display the species identification information of all samples at different taxonomic levels (phylum, class, order, family, genus, species), the software generates a Krona chart. As shown in Figure 29.

Figure29. Species distribution Krona diagram

The software can also annotate new functional genes in the samples. By comparing the new gene sequences with protein sequences in the COG database, it is possible to determine the potential functions and structures of the new gene sequences. This process has led to the discovery of some high-frequency known and unknown functional gene clusters, such as the S gene cluster, R gene cluster, and J gene cluster related to transcription, ribosomal structure, and synthesis, as shown in Figure 30.

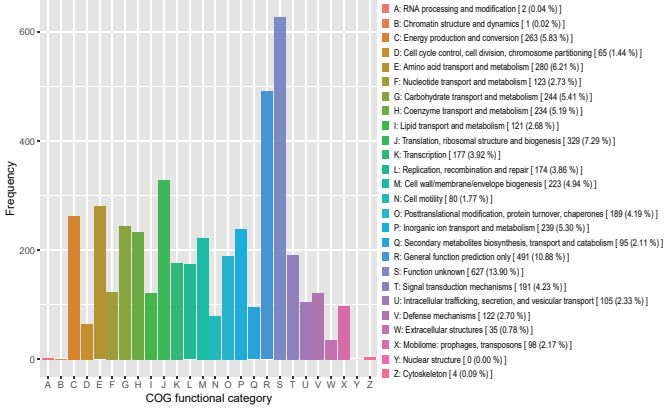

Figure30. Functional gene prediction diagram

In summary, the MGI Metabarcoding Metabarcode Sequencing Package compatible with a variety of sample types, has a wide range of application scenarios, excellent detection performance and powerful analytical capabilities. It is an ideal tool for microbiome research.

Ordering information

Type	Name	Specification	PN
Instrument	DNBSEQ-G99ARS	Include server	900-000609-00
	DNBSEQ-E25RS	Standard Config	900-000537-00
	Automated Nucleic Acid Extractor MGISP-NE32RS	——	950-000020-00
	MGISP-960RS High-throughput Automated Sample Preparation System	Configuration 9	900-000154-00
Reagent	MGIEasy Stool Microbiome DNA Extraction Kit II	96 Preps	940-001247-00
	MGIEasy Stool Microbiome DNA Extraction Kit II	384 Preps	940-001246-00
	MGIEasy Tissue Grinding Beads	96 Preps / Bottle	940-000136-00
	ATOPlex 16SV3V4 rDNA Library Preparation Set	96 Preps	940-001261-00
	ATOPlex 16SV3V4 rDNA Library Preparation Set	576 Preps	940-000725-00
	ATOPlex 16SV4 rDNA Library Preparation Set	96 Preps	940-002560-00
	ATOPlex 16SV4 rDNA Library Preparation Set	576 Preps	940-002559-00
	ATOPlex ITS1 rDNA Library Prep Set	96 Preps	940-002208-00
	ATOPlex ITS1 rDNA Library Prep Set	576 Preps	940-002200-00
	ATOPlex ITS2 rDNA Library Prep Set	96 Preps	940-002562-00
	ATOPlex ITS2 rDNA Library Prep Set	576 Preps	940-002561-00
	MGIEasy Dual Barcode Circularization Kit V1.0	16 RXN	1000020570
	DNBSEQ OneStep DNB Make Reagent Kit	4 RXN	1000026466
	ATOPlex E450 Dual Barcode Balanced Library Reagent	40 ng /Tube	940-000637-00
	Standard Library Reagent (PCR Product)	1500 ng / Tube	1000027585
	High-throughput Sequencing Set	G99 FCL PE150	940-001269-00
	High-throughput Sequencing Set	G99 App-D FCL PE300	940-001716-00
	DNBSEQ-E25RS High-throughput Sequencing Set	FCL PE150	940-000567-00
Software	Metabarcoding Species Identification Software	——	970-000417-00
	Metabarcoding Species Identification Software Package	96 reports	970-000456-00
	Platform of microorganisms Fast Identification	——	900-000393-00
	Platform of microorganisms Fast Identification and assembly evolution	——	900-000399-00

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