

The Microbiome Protocols eBook initiative: Building a bridge to microbiome research

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The microbiome is a research area that focuses on studying the omics of the microbe [1] with rapid development in the past few decades, making breakthroughs in understanding microbiological studies in humans [2], animals [3] and plants [4] as hosts, and the environments [5]. The concept of the microbiome has been extended to various fields. Thousands of microbiome articles are published every year, revolutionizing our conventional understanding of microbes in medicine, agriculture, and industry. At present, some standardized analysis software and protocols for microbiome studies have been developed. For example, QIIME2 [6] stands out as a popular integrated pipeline for amplicon sequencing data analysis, Minimum Information about any (x) Sequence (MIXS) serves as the standard for submitting microbiome sequences [7], Critical Assessment of Metagenome Interpretation [8] provides the standards of assessing metagenomics software. Despite these advancements in microbiome research, there remains a need for more standard methods for other types of microbiome data. In addition, few systematic and standard protocols for wet-lab experiments and data analysis were published, which impeded the progress of experiments or analysis in this area. To address these issues, we initiated the Microbiome Protocols eBook (MPB, <https://cn.bio-protocol.org/bio101/mpb>), aimed at providing a comprehensive resource for standardized wet-lab protocols in microbiome research.

The MPB was launched by Bio-protocol office in China and WeChat's official account "meta-genome," one of the largest microbiome communities with more than 164,000 subscribers (by February 2024). The Bio-protocol Journal offers peer-reviewed and open-access publications, at no cost. The MPB is designed to foster communications and

collaboration between researchers and research teams, with the goal of summarizing, sharing, and disseminating the wet-lab experiment protocols in the microbiome area. We anticipate that this project will bridge the gaps in microbiome protocols, addressing the challenges encountered in wet experiments and data analysis, while paving the way for accumulation of standard data for big data integrated analysis in the near future. In summary, the MPB is poised to essentially facilitate the progress of the microbiome area. All protocols are hosted in Bio-101, a companion website of the Bio-protocol Exchange. The project homepage link is <https://bio-protocol.org/bio101/mpb>.

The MPB encompasses a wide range of microbiome-related protocol, including culturomics [9], amplicon [10], metagenome, metatranscriptome, metavirome, metaproteome, metabolome, microbiome, related molecular biology and microbiology experiments. It also covers the upstream and downstream experimental protocols and analysis (Figure 1A, Supporting Information: Table S1). According to the research objects, it mainly includes the microbiome in humans, animals, plants, and the environment (Figure 1A, B). This resource comprehensively covers a range of research methods, it mainly includes sample preparation, nucleic acid extraction, protein and metabolite extraction, sequencing library preparation, microbial culture and identification, synthetic community, axenic system, data analysis, and general microbiology experiments and analysis. MPB is designed to be easily accessible to all, providing convenient access through many channels such as Bio-101, WeChat, Chinese Software Developer Network (CSDN, <https://www.csdn.net/>), and so forth. Its open-access nature ensures that anyone can explore and benefit from the latest protocols and techniques in microbiome research.

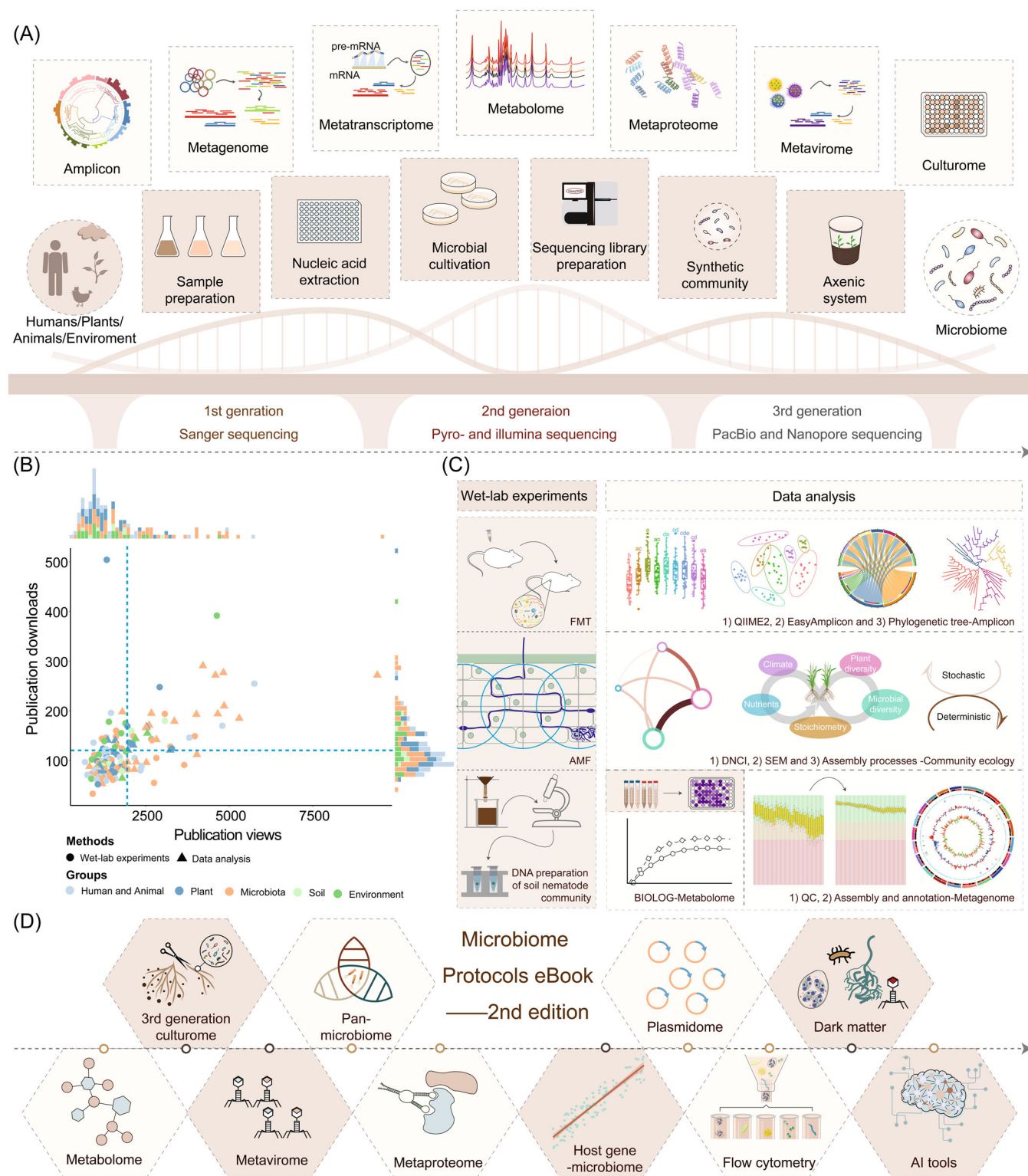


FIGURE 1 Microbiome Protocols eBook (MPB)—Building a bridge to microbiome research. The establishment and optimization of a variety of microbiome protocols make it possible to study the microbiome of humans, animals, plants, and the environment. (A) Sequencing technology, wet-lab experiments, and data analysis methods link the microbiome with its host or environment. (B) The publication views and downloads of each protocol published in MPB, until 19th January 2024. (C) The 12 most popular publications in MPB. (D) The 10 key recruitment focuses for the 2nd edition of the MPB. Vector for the 3rd generation culturome is modified from Zhang et al. [9]. AMF, arbuscular mycorrhizal fungi; DNCI, dispersal-niche continuum index; FMT, fecal microbiota transplantation; QC, quality control; SEM, structure equation modeling.

Since the first announcement of MPB in July 2020, 355 researchers from 125 institutes or universities have been involved in the MPB, including the Institute of Genetics and Developmental Biology, Institute of Soil Science, Institute of Microbiology, Research Center for Eco-Environmental Sciences, and Institute of Urban Environment of Chinese Academy of Sciences, Chinese Academy of Agricultural Sciences, Peking University, Tsinghua University, Zhejiang University, Sun Yat-Sen University, China Agricultural University, Shandong University, Yangzhou University, Westlake University, Nanjing Agricultural University, and so forth. As of January 19th, 2024, 1st edition of MPB has published 152 protocols, with an average of 1945.47 pageviews and 210.39 downloads in per protocol. Over the past year, microbiome data analysis has been particularly popular among readers (Figure 1B). Upon summarizing both pageviews and downloads data, we identified 12 most popular works, consisting of 3 wet-lab experiments and 9 data analysis works (Figure 1C, Supporting Information: Table S2). Notably, in previous endeavors, the mainstream focus remained on the data analysis of amplicon and metagenome, primarily due to the maturity of second-generation sequencing technology.

Of the 12 notable contributions highlighted in the 1st edition of MPB, several have significantly propelled research into microbiome analysis. For instance, the wet-lab experiment protocol of soil nematode community [11] has addressed the limitations of available nematode sequences, offering a standardized approach for studying soil nematode community using high-throughput techniques. Since then, it has garnered more than 3000 views and has facilitated the publication of five studies exploring the diversity or composition of soil communities. EasyAmplicon [10] has emerged as a widely utilized tool for amplicon data analysis. Since its publication in our protocol, it has accumulated over 4000 views. Our protocol serves as an interactive platform for both authors and users, fostering the continual improvement of the tool. To date, EasyAmplicon has supported 100 publications for mining data, spanning the microbiome in animal, soil, waste water, plant, wine, and so forth.

To ensure the quality, diversity, and timeliness of the MPB, we have established it as a long-term project with biennial updates. In the upcoming 2nd edition of MPB, we are excited to incorporate updates from the 1st edition's protocols while also expanding into new areas, covering innovative methodologies and emerging technologies. This includes aspects such as the 3rd generation culturome, pan-microbiome [12], metabolome [13], metavirome, metaproteome, plasmidome, the "dark matter" of the microbiome [14], and the interaction of host genetics with the microbiome (Figure 1D). Additionally, we will feature

cutting-edge technologies and data algorithms, including flow cytometry and AI tools, such as machine learning and deep learning algorithms (Figure 1D). The published protocols can be accessed on the project homepage. We sincerely invite more researchers to participate in this project and contribute to their protocols. Any protocols related to the microbiome are welcome, especially for the commonly used or cutting-edge protocols related to the 10 key focuses (Figure 1D). We hope MPB becomes a protocol encyclopedia and a valuable tool for microbiome research.

AUTHOR CONTRIBUTIONS

Yong-Xin Liu, Yang Bai, and Haiyan Chu conceived and coordinated this work. Yunyun Gao and Kai Peng authored the paper, and the other authors have revised the manuscript. All authors have read the final manuscript and approved it for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. All the protocols are open access in <https://bio-protocol.org/bio101/mpb>. Supplementary materials (tables, graphical abstracts, slides, videos, Chinese translated version and update materials) may be found in the online DOI or iMeta Science <http://www.imeta.science/>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. The publication views and downloads of each protocol published in the first edition of MPB, until 19th Jan, 2024.

Table S2. The 12 most popular publications in the first edition of MPB.

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