

Metagenomics Analysis of Microbiota by Next Generation Shotgun Sequencing

Understand the genetic potential of your community samples
Provides you with hypothesis-free taxonomic analysis

Introduction

Microbiome studies are often based on the sequencing of specific marker genes as for instance the prokaryotic 16S rRNA gene. Such amplicon-based approaches are well established and widely used. However, they have limitations such

as the dependency on a single gene to analyze a whole community, the introduction of PCR bias and the restriction to describe only the taxonomic composition and diversity. Shotgun metagenomics is a cutting edge technique for

microbiome analysis overcoming said limitations. Whole genomic DNA of a sample is isolated, fragmented and finally sequenced. This allows a detailed analysis of the taxonomic and functional composition of a microbial community.

Microsynth Competences and Services

Microsynth offers a full shotgun metagenomics service for taxonomic and functional profiling of clinical, environmental or engineered microbiomes. The service covers the entire process from experimental design, DNA isolation, tailored sequencing to detailed and customized bioinformatics analysis. A major advantage of Microsynth's shotgun metagenomics service is the project specific consulting and support by our experienced NGS specialists.

Experimental Design: Microbiome profiling by shotgun metagenomics often means sequencing of numerous samples generating millions or even billions of reads. To gain reliable and high-quality data, a well-planned experimental design including replicates and a clear scientific hypothesis is essential [1]. Our NGS specialists will assist you from the beginning.

DNA Isolation: Total DNA is either isolated by the customer or this critical step is outsourced. Microsynth has over 15 years of experience in nucleic acid isolation from various demanding matrices such as food, stool, eukaryotic tissues (plants, animals, human), water or soil.

Tailored NGS Sequencing: Microsynth will tailor the whole sequencing process to your project requirements, thus providing you with just the right amount of

data to answer your questions.

Bioinformatics: Taxonomic and functional analysis of metagenomic datasets is challenging. Alignment, binning and annotation of the large amounts of sequencing reads require expertise and sufficient computational power. Microsynth offers cutting edge bioinformatics analysis for your shotgun sequencing data using published and well-established methods. Our analysis pipeline will be adjusted to your specific requirements to guarantee scientifically reliable results for your project. After quality processing the reads are aligned against a protein reference database

(e.g. NCBI nr) using DIAMOND, a sensitive tool 20,000 times faster than BlastX [2]. Taxonomic and functional binning and annotation are performed by MEGAN [3]. The analysis is not restricted to prokaryotes but also includes eukaryotes and viruses. The output is one file per sample containing all reads, alignments as well as the taxonomic and functional annotation. Together with the initial FASTQ file containing the raw sequencing data, the whole microbiome is represented by just two files per sample. The microbiome can be explored and analyzed by using the freely available community edition of MEGAN.



Microbiome Data Analysis Examples

Microsynth's analysis pipeline provides you with full flexibility regarding data inspection and analysis. It allows you to analyze and visualize the taxonomic and functional composition of your microbiome dataset in a single user friendly interface by using MEGAN. A major advantage is the possibility to import metadata such as environmental parameters or experimental conditions for further statistical analysis. On all levels, data can easily be exported in prevalent formats, giving you the possibility to specifically extract the relevant information from your dataset and use the data for downstream analysis and figure generation. Taxonomic content can be explored on all ranks from domain level

down to the species level covering the whole NCBI taxonomy (see **Figure 1**). Functional annotation is based on different functional classification systems such as InterPro, KEGG, eggNOG or SEED. All systems contain a functional hierarchy ranging from general classifications such as metabolism or cellular functions (see

Figure 2) down to single enzymes and subunits. Beside the analysis of single samples, multiple sample comparisons are possible (see **Figure 3**). Samples can be grouped and metadata information can be included in the analysis. Various beta diversity measures can be chosen for sample comparison.



Figure 1. Word cloud representing the bacterial composition on the phylum level in a sample. The size of the words is proportional to the number of assigned reads.

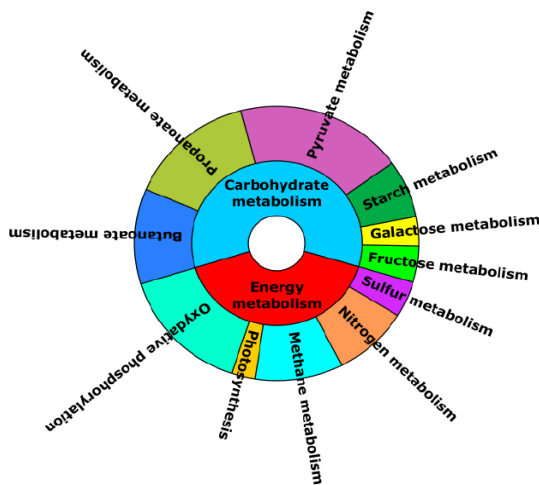


Figure 2. Radial tree chart representing the KEGG functions carbohydrate metabolism and energy metabolism and the related subgroups. The size of the sections in each hierarchy is proportional to the number of assigned reads.

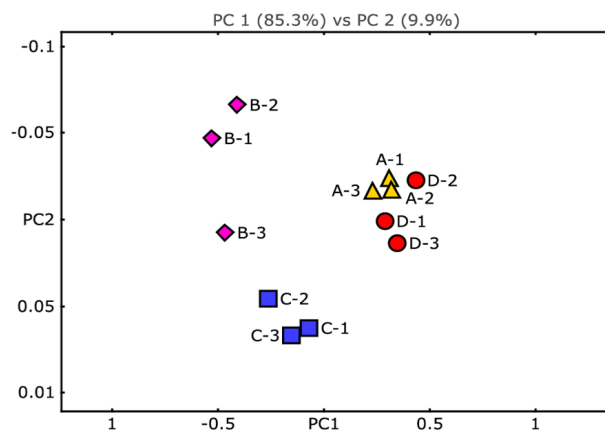


Figure 3. PCoA for multi-sample comparison based on Bray-Curtis dissimilarities for the InterPro functional annotation.

Related Topics

- 16S Metagenomics Sequencing
- Amplicon Deep Sequencing
- Bioinformatics Services
- DNA/RNA Isolation

Further Reading

1. Knight et al. (2012) Unlocking the potential of metagenomics through replicated experimental design, *Nat Biotechnol*, 30, 513-520.
2. Buchfink et al. (2015) Fast and sensitive protein alignment using DIAMOND, *Nat Methods*, 12, 59-60.
3. Huson et al. (2016) MEGAN Community Edition – Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data, *PLOS Comput Biol*, 12, e1004957