Abstract

Metagenomics has many applications in different fields such as medicine, engineering, agriculture among others, as well as, benefits from DNA sequencing technology improvements and the continuous development and evolution of dedicated bioinformatics tools and databases. However, there is no universally suitable analysis strategy that guarantees best results for all applications. Experimental design, different input data and hypothesis require detailed knowledge about used tools, databases and possible combinations of the analysis steps. This is a major challenge for non bioinformatics experts.

Here the novel Metagenomics Module of OmicsBox is presented. It employs best practices in an intuitive way with many analysis options. It works in an out-of-the-box way without complex installations or specific hardware requirements. Up-to-date tools and databases are directly available via OmicsBox cloud. Raw read sequencing data, either 16S amplicon based or shotgun data can be used. Analysis steps can be combined in many possible ways and custom pipelines can be created with a workflow editor. This allows to obtain biological insights in a timely manner. All analysis steps can be carried out on a standard laptop or workstation with 4GB of RAM running Windows, Linux or macOS.

Workflows

Taxonomic Classification

The workflow in the figure on the right shows taxonomic classification of metagenomics data with OmicsBox. First, the reads are preprocessed, and a report is generated. Kraken is used to identify and count all the different OTUs for further interpretation. The spreadsheet-like result can be filtered and organized. The PDF report gives a clean overview about the most abundant OTUs at different levels for each sample.

Functional Annotation

Functional characterization of metagenomics data is a complex task. The OmicsBox Metagenomics module allows you to design streamlined workflows to easily combine the typically resource-demanding assembly step with gene prediction, as well as high-throughput functional annotation for large metagenomics datasets. The workflow in the bottom figure shows an example workflow of the combination of MEGAHIT with Prodigal. Fast and comprehensive functional annotation is achieved with the integration of EggNOG-Mapper and PfamScan.

OTU Abundances Table

Sort and filter the taxonomic classification results, and extract all reads that belong to selected OTUs. Continue the assembly with reads that belong to selected groups of OTUs and obtain a more specific assembly. Refine your reports with customized distribution charts in various styles.

OTU Abundances Pie Chart

Inter-Sample Comparison Bar Chart

Datasets

16S and WGS Metagenomics

Quality Control and Assessment

FastQC, Trimmomatic

Taxonomic Classification

Kraken + Custom DB

MEGAHIT, meta-SPAdes

Gene Prediction

FragGeneScan, Prodigal

Functional Annotation

EggNOG-Mapper, PfamScan, Blast2GO

Comparative Analysis

OTU Table, Krona, Bar Chart

Comparative Analysis

Comparison Charts, GO Graphs, GOSlim

Colored GO Graph

Comparison annotate abundances visually between samples and take into account the GO hierarchy. Nodes are colored partially depending in which samples they are more abundant.

Results

Some of the major features of the OmicsBox Metagenomics Module include:

- **Inter-Sample Comparison Bar Chart**
- **OTU Abundances Table**
- **Software Selection**
- **Functional Abundance Comparison**
- **Colored GO Graphs**
- **Comparison Analysis**