Microsynth

Metatranscriptome Analysis by Next Generation Shotgun Sequencing

Understand the active genetic composition of your samples
Functional results for active transcripts and switch on pathways

Introduction

Shotgun metatranscriptomics is emerging as a powerful technology for the functional characterization of complex microbial communities (microbiomes). While metagenomics tells us which microbes are present and what genomic potential they have, metatranscriptomics tells us about their activity: the (differential) expression of genes (mRNA transcripts) in a specific microbial environment at a given point of time. However, the lack of established reference genomes, computational tools and bioinformatics pipelines make analysis and interpretation of large sequencing datasets challenging. Microsynth has become an expert in this area and can help you in any aspect of a metatranscriptomic sequencing project. Therefore, approach us in case your application includes one or several of the following topics:

- Determination of gene expression profiles in microbial community across all species
- Identification of community activities and functions, e.g. active biochemical functions and metabolic pathways
- Discovery of novel genes and regulatory pathways
- Differential gene expression analysis, e.g. detection of functional changes in a given context

Microsynth Competences and Services

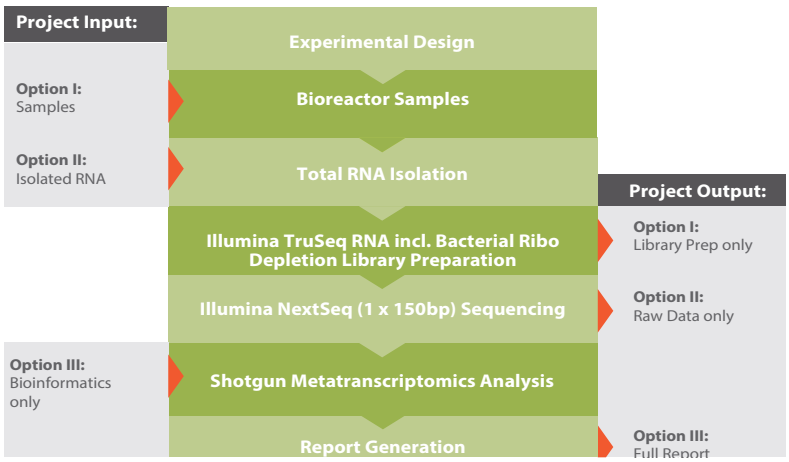
Microsynth offers a complete shotgun metatranscriptomics service for taxonomic and functional profiling of clinical, environmental or engineered samples. The service covers the entire process from experimental design, RNA isolation, tailored sequencing to detailed and customized bioinformatics analysis.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

Experimental Design: Gene expression profiling by shotgun metatranscriptomics often means sequencing of numerous samples generating millions or even billions of reads. To gain reliable and highquality data, a well-planned experimental design including replicates as well as a clear scientific hypothesis is essential. Our NGS specialists are happy to assist you from the very beginning.

Sampling: For each metatranscriptome analysis, sampling is the most critical step. Since the analysis will result in a snapshot of the gene expression profile at the time of sampling, adequate sampling procedures are essential for obtaining an unbiased gene expression profile.

RNA Isolation: Total RNA 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, water or soil.

Library Preparation and Sequencing: Since a large fraction of total RNA in a cell accounts for ribosomal RNA, depletion of ribosomal RNA or poly(A) enrichment are optional steps after RNA isolation to increase the fraction of coding RNA. Library preparation includes reverse transcription of RNA into cDNA and preparation of Illumina shotgun libraries. Microsynth will tailor the whole sequencing process to your project



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requirements, thus providing you with just the right amount of data to answer your questions.

Bioinformatics Analysis: Taxonomic and functional analysis of metatranscriptomic datasets is challenging. Alignment, binning and annotation of 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. Furthermore, our analysis pipeline will be adjusted to your specific project requirements in order to yield scientific reliable results. The reads are quality processed and aligned against a protein reference database (e.g. NCBI nr) using DIAMOND, a sensitive tool 20,000 times faster than BlastX [1]. Taxonomic and functional binning and annotation are performed by MEGAN [2]. The analysis is not restricted to prokaryotes but also includes eukaryotes and viruses. The number of reads mapped to an annotated feature (e.g. gene IDs) is the input for the differential gene expression analysis. Specialized statistical software packages are used to identify differentially expressed genes. First, data is normalized and variance is calculated based on replicate samples for each condition. Finally, differentially expressed genes are identified by statistical testing.

Examples for Metatranscriptome Data Analysis

Microsynth's analysis pipeline provides you with full flexibility regarding data inspection and analysis. The metatranscriptome can be explored and analyzed using the freely available community edition of MEGAN. On all levels, data can easily be exported in standard formats giving you the possibility to specifically extract the relevant information from your dataset and use the data for downstream analysis and figure generation. Differential gene expression analysis results in a log fold change per gene for the pairwise comparison between two conditions as displayed in ***Figure 1A***. Examples for graphical output are heatmaps of the top up- and down-regulated genes (see ***Figure 1B***). For more analysis examples, please see also our Application Note "Metagenomics Analysis of Microbiota by Next Generation Shotgun Sequencing" (please click here to be directed to our website section where you can download the corresponding PDF).

A

Condition1 vs Condition2

5 records per page Search all columns:

ID	Image	From to logFC	From to p-Value	From to Adjusted p-Value
IPR000100		-2.510	3.57e-13	7.39e-12
IPR001469		-2.540	3.58e-13	7.39e-12
IPR013461		-2.510	5.37e-13	1.10e-11
IPR000406		-5.990	6.35e-13	1.30e-11
IPR024922		-2.860	7.00e-13	1.42e-11

B

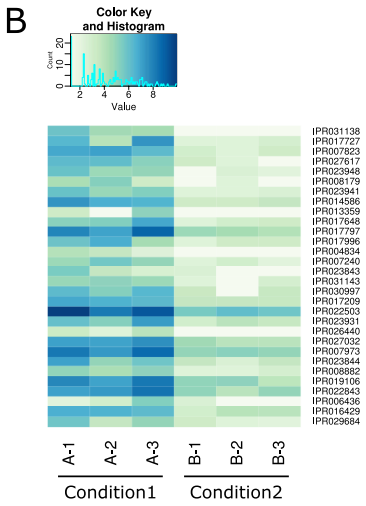

Figure 1: *Results from the metatranscriptome analysis. 1A. Summary table resulting from differential gene expression analysis of the metatranscriptomic dataset. The IDs refer to the InterPro classification for proteins. The column logFC represents the logarithmic fold change between Condition1 and Condition2. Statistical significance is given by the p-value and the adjusted p-value for multiple testing. 1B. Heatmap of the top 30 upregulated genes between Condition1 and Condition2.*

Related Topics

- Shotgun Metagenomics
- RNA Sequencing
- 16S Metagenomics Sequencing
- Bioinformatics Services

Further Reading

1. *Buchfink et al. (2015) Fast and sensitive protein alignment using DIAMOND, Nat Methods, 12, 59-60.*
2. *Huson et al. (2016) MEGAN Community Edition – Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data, PLOS Comput Biol, 12, e1004957.*