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Microbial profiling analysis report**Authorization**

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1. Introduction

This report contains a detailed overview of the analysis and interpretation of the sequencing results of your Next Generation Sequencing-based 16S or ITS microbial profiling experiment

This report contains the following sections:

1. Introduction (this paragraph)
2. OTU classification pipeline
3. Metagenomics online analysis portal
4. Availability of raw sequence files (Illumina FASTQ)

2. OTU classification pipeline

Short sequence reads were generated using the Illumina MiSeq platform. Assignment of sequence reads into Operational Taxonomic Units (OTUs) was performed using a proprietary pipeline. Classification of bacterial organisms is based on a combination of 16S gene databases. Classification of fungal organisms is based on the UNITE ITS gene database (Abarenkov et. al, 2010). The end result is a list of OTUs and corresponding frequencies. Further details of the pipeline and procedure are provided in the Metagenomics online portal manual.

3. Metagenomics online analysis portal

The results of the taxonomic classification can be inspected through an interactive online platform, which is a very easy way of analysing a metagenomics community. The platform requires no additional software installation and can be readily accessed through <https://genome-explorer.com/> using the following login details:

- Username: J_Bouwman
- Password: 9pNHR5yGV4

The platform contains a number of unique features among which:

- Phylogenetic tree and OTU bar-chart providing a general overview of the taxonomic composition in all samples.
- Table containing for each sample the assigned taxonomies and their relative abundance.
- Pie-chart (KRONA) which allows interactive interpretation at user-defined taxonomic levels.

Note that all tables and figures can be exported in multiple formats. A free license for the portal is offered for a period of one year (starting on the delivery date mentioned in this report).

Further details of the online platform and related options are provided in the Metagenomics online portal manual.

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4. Availability of raw sequence files (Illumina FASTQ)

Your data and analysis results can be downloaded in two ways:

| Order placed via order portal? | Data available by: | |
|--------------------------------|---|---|
| | Order portal (link) | FTP's server |
| Yes | Yes, use username/password combination for order portal | Yes, use username/password combination for order portal |
| No | - | Username: XXX Password: XXX |

The secure FTP server can be accessed using a FTP's client (e.g. FileZilla). After installation, open the program and login with your username/password combination.

FTP connection details:

Host/port: ftp.baseclear.com / 990
Protocol: FTP – File Transfer Protocol
Encryption: Require implicit FTP over TLS

Important notes:

- The data will be available on our FTP server for the coming month.
- Downloads via SFTP (SSH File Transfer Protocol) are **not** supported at this time.

Quality analysis of FASTQ sequence reads

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Quality analysis of FASTQ sequence reads

Quality statistics after CASAVA and FastQC analysis

| Samples | Number of reads | Sample Yield (in MB) | Average Quality scores (Phred) |
|-------------------|-----------------|-------------------------|-----------------------------------|
| ILC-oral-1 | 72,697 | 43 | 34.67 |
| ILC-oral-2 | 74,142 | 44 | 33.36 |
| ILC-oral-3 | 81,206 | 48 | 34.56 |
| ILC-oral-4 | 62,084 | 37 | 34.16 |
| ILC-oral-5 | 73,715 | 44 | 33.41 |