

# Hands-on 16S rRNA gene amplicon analysis seminar

2021 April, the 12-14<sup>th</sup>

*Technische Universität München, ZIEL - Institute for Food & Health*

Supported by the NuGO association, two NuGO member's affiliates, Giorgia Secchi from Fondazione Edmund Mach (Tn), and Laura Bordoni, University of Camerino, attended the online seminar on 16S rRNA gene amplicon analysis organized by the Technical University of Munich from 12<sup>th</sup> to 14<sup>th</sup> of April, 2021.

The workshop started with an introduction about amplicon-based next generation sequences, with a specific focus on 16S rRNA for microbiome analysis. Critical points to be considered from the initial steps of the study design have been described. An exhaustive description of how to measure richness,  $\alpha$ -diversity and  $\beta$ -diversity and the different indexes associated to these parameters has been illustrated. These general concepts were requested to fully understand and properly handle the IMNGS (Integrated Microbial NGS platform) and Rhea scripts proposed for the analysis of microbial profiles.

Giorgia and Laura have learnt how to process raw sequencing data (in the format of FASTQ files) from the quality check (with FASTQC application) to the production of remultiplex from dimultiplex files (using Perplexor scripts). Then, they have learnt how to produce OTU (Operational Taxonomic Units) tables with IMNGS and further analyse them with Rhea scripts. These scripts represent an easy tool suitable for normalization and rarefaction curves production,  $\alpha$ -diversity and  $\beta$ -diversity analysis, taxonomic binning, serial group comparisons and correlation analysis. The course provided both a theoretical background on these topics (with daily morning lectures) and opportunities to have practice with the mentioned pipeline (with separated hands-on sessions in the afternoon). These practical sessions included also an overview of advanced analysis (with Namco) and how to produce figures for high quality papers in this research field, depending on the number of samples, the presence of confounders or in case of longitudinal data, for an effective data communication and to avoid misinterpretations.

Despite the restrictions due to the COVID-19 pandemic, this online course represented a wonderful learning opportunity to keep educating young researchers of the NuGO network.