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| **Title** | **Metabarcoding Pipeline Building** |
| **Dates** | 20 to 24 April 2020 |
| **Organizer(s)** | Rachel Korn, UNIFRDr Magdalena Steiner, Agroscope Wädenswil  |
| **Speakers** | Prof. Gerhard Thallinger, Technical University of GrazRachel Korn, UNIFRDr Magdalena Steiner, Agroscope Wädenswil  |
| **Description** | Metabarcoding is a rapidly evolving technique that identifies DNA from a set of often unknown organisms and reveals microbial communities in unprecedented taxonomical resolution by using universal PCR primers and high-throughput sequencing. It increasingly gains importance in almost all fields of biological and medical sciences. Its wide application range includes biodiversity assessment and monitoring, diet analysis, reconstruction of paleocommunities and more. The processing of metabarcoding data requires a broad range of skills at the interface of molecular biology, bioinformatics, biostatistics and biology/ecology.In this workshop the participants will be introduced to the workflow of microbiome research targeting the 16S and 18S rRNA gene: preceded by an overview of the full process, this course is aimed at the analysis of high-throughput sequencing data with open-source and cross-platform software (mothur and R/bioconductor packages) from the raw reads to the visualization of results. Theoretical content will be visualized and strengthened by practical exercises. Participants will practice during the workshop on prepared data sets.***Prior requirements***A basic statistic knowledge as well as basic skills in R are a precondition for this workshop. The participants are required to bring their own notebooks with the software already installed. For this, a Docker image containing all necessary software will be provided to guarantee architecture and version control.**Schedule (preliminary)*****Introduction to metabarcoding**** DNA extraction
* Internal controls (positive, negative)
* Primer choice (16S, 18S, ITS…)
* NGS technologies (Illumina, IonTorrent, PacBio)
* Sequencing variants, data formats
* FASTQ format
* Reference databases (SILVA, greengenes, PR2, …)
* Amplicon sequence variants (ASV/ESV) vs. operational taxonomic units (OTU)

***Quality control**** Visualization, trimming and filtering (dada2)

***Pipeline: OTU construction (mothur)**** Read merging/contigs assembly
* Alignment to reference database (SILVA)
* Pre-clustering
* Chimera detection
* Singleton filtering
* Distance matrix
* OTU clustering, generation and classification (taxonomy, methods)

***Pipeline: ASV construction (dada2)**** Denoising (sample-wise error rate)
* Read merging/contigs assembly
* Chimera detection
* Taxonomy assignment (SILVA, DECIPHER)

***Analysis (R, main packages: phyloseq & vegan)**** BIOM format
* Analysis approaches: OTU/ASV vs. phylogeny
* Preprocessing & vizualisation (absolute, relative, rank abundance)
* Taxonomic filtering
* Rarefaction
* Community composition (α-, β- and γ-diversity, diversity indices...), multivariate analysis (ordination)
* Differentially abundant OTUs/ASVs
* LefSe (Linear discriminant Effect Size analysis)
* Procrustes
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| **Location** | University of Fribourg, Boulevard de Pérolles 90, building 21/22, rooms G314 & G514, 1700 Fribourg |
| **Evaluation** | Full attendance and active participation.**Make sure to sign the attendance list each and every day!** |
| **Information** | **When?**20 to 24 April 2020, 9:00 to 12:00 and 13:30 to 17:00**Where?**University of FribourgBoulevard de Pérolles 90, building 21/22, rooms G314 & G5141700 Fribourg*The course is organized in collaboration with the CUSO Microbiology, CUSO StarOmics and UNINE Organismal Biology Doctoral Programs.*16 places in total (4 for each program) |