Microbial community analysis

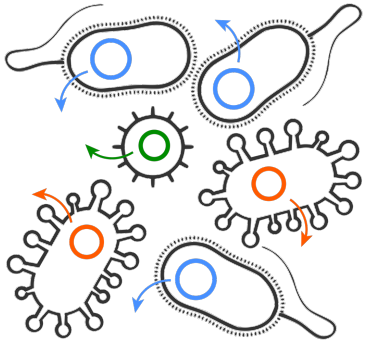
DNASense

- Microbial communities affect all aspects of human activity, and understanding their significance requires high resolution community analysis
- Analysing the structure and species abundances of microbial communities is possible by sequencing taxonomic marker genes (e.g. 16S rRNA and ITS genes)

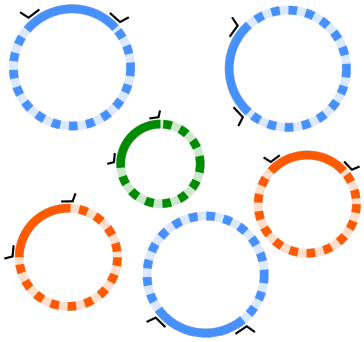
DNASense provides sample-to-answer services for gene sequencing and microbial community analysis

DNA extraction and sequencing

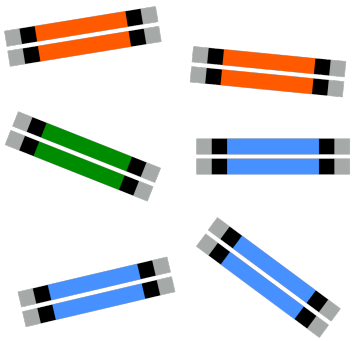
DNA extraction



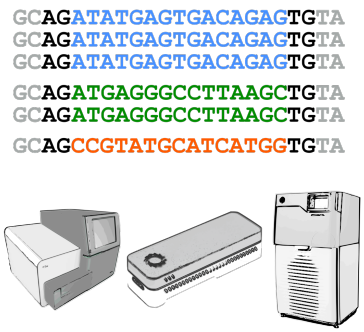
Gene amplification (PCR)



Library preparation (PCR)

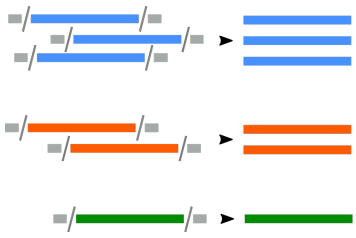


DNA sequencing

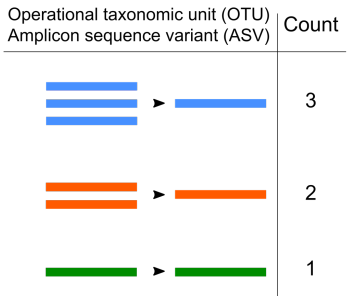


Cutting-edge bioinformatic analysis

Trimming & quality filtering

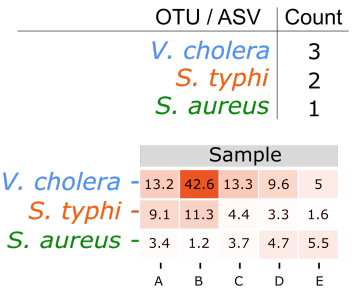


Sequence clustering

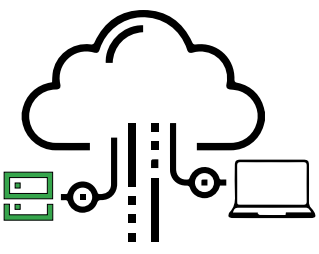


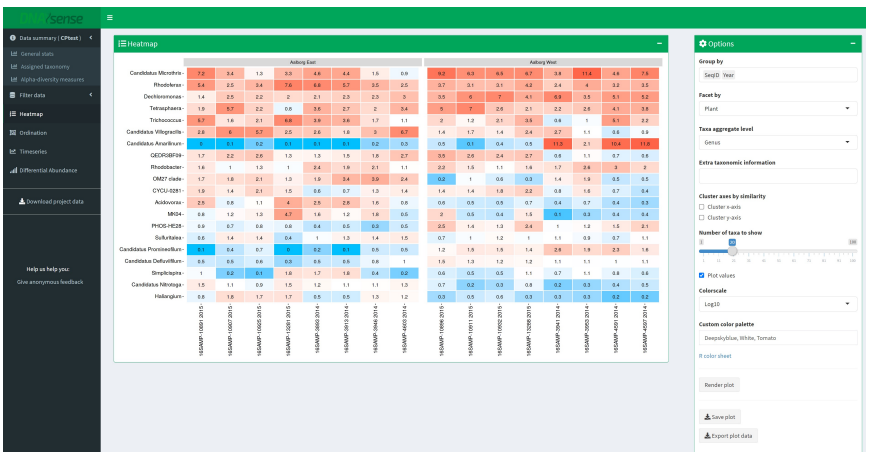
Taxonomy assignment

Analysis & visualization



Access and explore results online





with video [tutorials]

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Working with the DNASense team

- Extensive experience from hundreds of projects and challenging samples
- Detailed documentation and full method transparency
- State-of-the-art sample preparation, DNA sequencing and bioinformatics
- Extensive expert consultant services

Encompassing report with actionable results

2.3 Microbial community composition

Figure 3 gives an overview of the 20 most abundant genera across all samples or sample groups and arranged by sampleGroup.

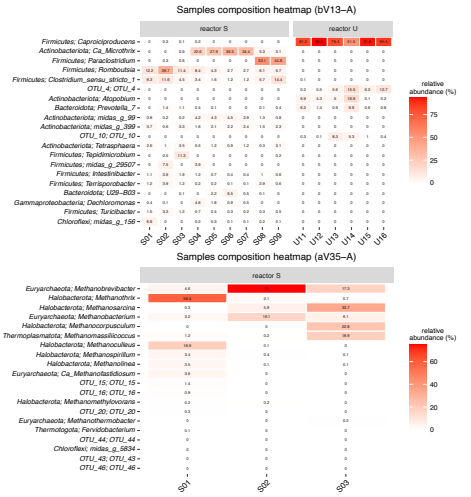

Figure 3: ***Heatmap of the 20 most abundant genera.*** *The most abundant genera in all samples arranged by sampleGroup. Where available the OTU's phylum classification is provided along with genus, and if no genus level classification could be obtained, the lowest assigned taxonomic classification is given. Values are shown as normalised fraction of total sequences (%).*

Figure 4 shows comparison of overall microbial compositions using multivariate statistics (PCA) for all samples.

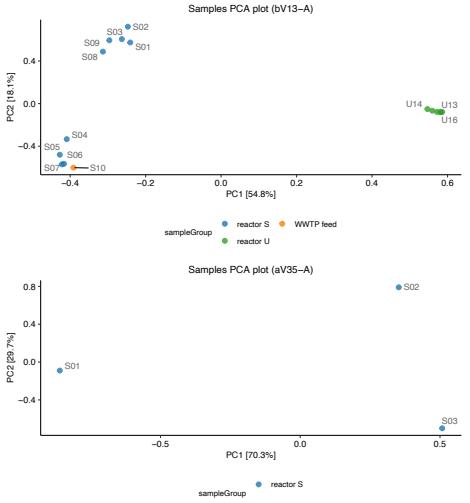

Figure 4: ***Principal component analysis (PCA).*** *Identification of samples with similar microbial communities using multivariate statistics. Each point represents the microbial community in a specific sample. Distance between the sample dots signifies similarity; the closer the samples are, the more similar microbial composition they have.*

3 Materials and methods

The project data analysis and reporting was done using DNASense's custom bioinformatic workflow (version *MCA_DS211020*).

3.1 Sample DNA extraction

3.1.1 FastDNA SPIN Kit for Soil

DNA extraction of samples was done using a slightly modified version of the standard protocol for FastDNA Spin kit for Soil (MP Biomedicals, USA) with the following exceptions. 500 µL of sample, 480 µL Sodium Phosphate Buffer and 120 µL MT Buffer were added to a Lysing Matrix E tube. Bead beating was performed at 6 m/s for 4x40s (Albertsen et al., 2015). Gel electrophoresis using Tapestation 2200 and Genomic DNA screentapes (Agilent, USA) was used to validate product size and purity of a subset of DNA extracts. DNA concentration was measured using Qubit dsDNA HS/BR Assay kit (Thermo Fisher Scientific, USA).

3.2 Sequencing library preparation

Sequencing libraries for the bacteria 16S rRNA gene variable regions 1-3 (bV13-A) were prepared using a custom protocol based on (Caporaso et al., 2012). Up to 10 ng of extracted DNA was used as template for PCR amplification of the bacteria 16S rRNA gene variable regions 1-3 (bV13-A) amplicons. Each PCR reaction (25 µL) contained 12.5 µL PCRBIO Ultra mix and 10 µL barcoded library adaptors (400 nM of each forward and reverse). PCR was done with the following program: Initial denaturation at 95 °C for 2 min, 25 cycles of amplification (95 °C for 15 s, 55 °C for 15 s, 72 °C for 50 s) and a final elongation at 72 °C for 5 min. Duplicate PCR reactions were performed for each sample and the duplicates were pooled after PCR. The adaptors contain the primers targeting the bacteria 16S rRNA gene variable regions 1-3 (bV13-A): [27F] AGAGTTTGATCCTGGCTCAG and [534R] ATTACCGCGGCTGCTGG (Ward et al., 2012).

Amplicon libraries for the archaea 16S rRNA gene variable regions 3-5 (aV35-A) were prepared by a custom protocol based on an Illumina protocol (Illumina, 2015). Up to 10 ng of extracted DNA was used as template for PCR amplification of the archaea 16S rRNA gene variable regions 3-5 (aV35-A) amplicons. Each PCR reaction (25 µL) contained (12.5 µL) PCRBIO Ultra mix and 400 nM of each forward and reverse tailed primer mix. PCR was done with the following program: Initial denaturation at 95 °C for 2 min, 30 cycles of amplification (95 °C for 15 s, 55 °C for 15 s, 72 °C for 50 s) and a final elongation at 72 °C for 5 min. Duplicate PCR reactions were performed for each sample and the duplicates were pooled after PCR. The forward and reverse, tailed primers were designed according to (Illumina, 2015) and contain primers targeting the archaea 16S rRNA gene variable regions 3-5 (aV35-A): [Arch-340F] CCCTAHGGGGYGCASCA and [Arch-915R] GWGCYCCCCCGYCAATTC (Pinto and Raskin, 2012)). The primer tails enable attachment of Illumina Nextera adaptors necessary for sequencing in a subsequent PCR. The resulting amplicon libraries were purified using the standard protocol for CleanNGS SPRI beads with a bead to sample ratio of 4:5. DNA was eluted in 25 µL of nuclease free water. Sequencing libraries were then prepared from the purified amplicon libraries using a second PCR. Each PCR reaction (25 µL) contained PCRBIO HiFi buffer (1x), PCRBIO HiFi Polymerase (1 U/reaction) (PCRBiosystems, UK), adaptor mix (400 nM of each forward and reverse)

Download example report here and explore results in the DNASense app

(user **EP001** and password: **quite75councils**)

DNA extraction	Sequencing	Raw data + QC	Analysis + app access	Price/sample (€ excl. VAT)		
				≥ 10 samples	≥ 50 samples	≥ 100 samples
■	■	■	■	140	120	105
	■	■	■	125	105	95
■	■	■		110	95	85
	■	■		100	85	75

- standard turn-around-time is 5 workweeks
- 9 workdays fast-track available (1.200 € fee)
- Many add-on services and customised workflows available

Founded in 2014, DNASense makes cutting-edge DNA sequencing and bioinformatics readily available to life science research in industry and academia