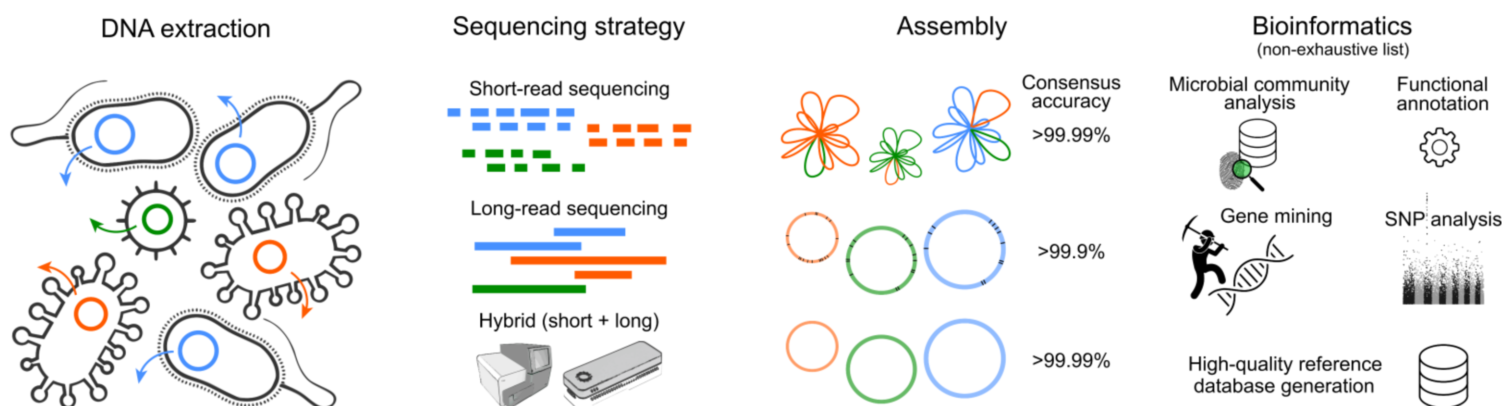
Genomics and Metagenomics analysis

DNASense

- Genomes from pure culture isolates or metagenome assembled genomes (MAGs) from complex samples provide valuable insight into the metabolic potential.
- A whole-genome based analysis potentially offers higher taxonomic resolution and less biased estimation of the microbial abundance.

DNASense provides sample-to-answer services for whole genome-based *de novo* assembly, genome binning and downstream functional characterisation

DNA extraction and sequencing

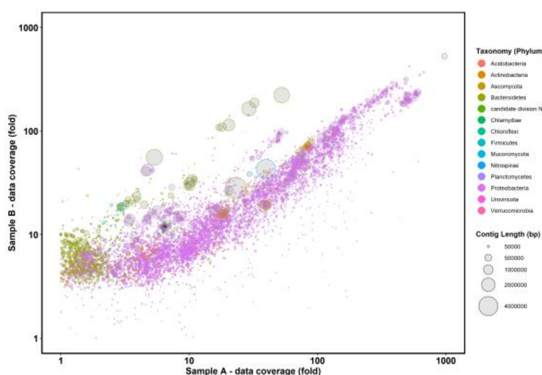


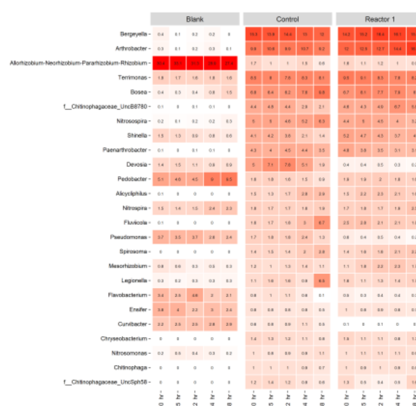
Cutting-edge bioinformatic analysis

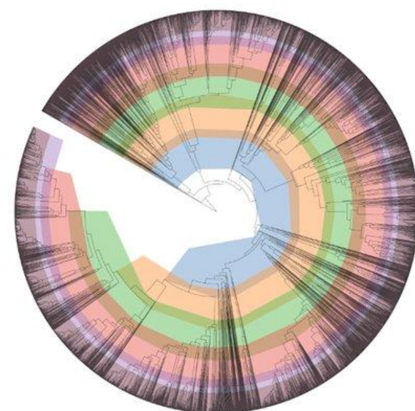
Genome and metagenome *de novo* assembly

Microbial community analysis

Phylogenetic analysis







Our standard package includes: Optional pre- and post-project meeting with a DNASense specialist, DNA extraction, library preparation, sequencing, pre- and post-sequencing quality control, de novo assembly, taxonomic profiling, gene annotation, online-access to raw data and result files and a detailed project report.

Add-on services (non-exhaustive list): Tailored DNA extraction and purification, genome binning, SNP-calling, Functional annotation (KO, GO and KEGG), functional enrichment analysis, manual curation of metabolic pathways, gene mining, core-genome SNP analysis, multi-locus sequencing typing (MLST), custom gene annotation, epigenetic analysis, data submission.

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Working with the DNASense team

DNASense

- 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

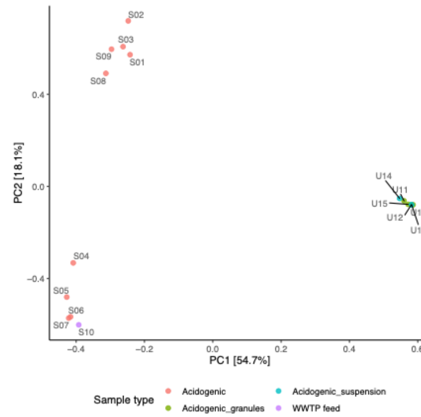
3.2 Table 2: The 25 most abundant genera in samples S01-S10

The most abundant genera across all samples. If no genus level classification could be obtained, the lowest assigned taxonomic classification is given. In addition, the phylum level classification is given (Proteobacteria at class level).

	Reactor S									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
Actinobacteria; Candidatus Microthrix	0	0	0.9	22.5	28	36.6	34.5	5.3	3	46.6
Firmicutes; Peptoclostridium	1.2	4.8	1.9	0.2	0.1	0	0	53	45.2	0
Firmicutes; Intestinibacter	13.2	42.6	13.3	9.6	5	3.1	3.1	7.1	6.3	1.9
Firmicutes; Clostridium sensu stricto 1	9.1	11.3	4.4	3.3	1.6	1.2	1.3	5.3	14.2	0.8
Actinobacteria; Tetrasphaera	3.4	1.2	3.7	4.7	5.5	5.5	5.1	1.5	3.9	4.7
Firmicutes; Clostridium sensu stricto 12	0	8.2	0.3	5.2	0.7	1.1	0.8	0.7	0	0
Actinobacteria; f__PeM15_OTU_45	1.5	0.3	1.4	1.3	1.8	1.9	2	1	1.1	1.9
Firmicutes; Tepidimicrobium	0	0.5	11.3	0	0	0	0	0	0.2	0
Firmicutes; Terrisporobacter	1.2	4.2	1.4	0.3	0.2	0.1	0.1	3	0.6	0.1
Chloroflexi; T78	8.8	0	0	0.2	0.3	0.1	0.1	0.2	0.1	0.1
k__Unassigned_OTU_55; k__Unassigned_OTU_55	0	0	0.1	0	2.2	6.5	0.5	0.1	0	0
Chloroflexi; Candidatus Defluviifilum	0.6	0.1	0.1	1.1	1.4	1.6	1.7	0.2	0.4	1.7
Actinobacteria; Ornithinicoccus	0.1	0	0.1	1.4	1.8	1.5	1.8	0.3	0.3	1.3
Firmicutes; Turicibacter	1.4	3.3	1.2	0.7	0.4	0.3	0.2	0.3	0.5	0.1
Betaproteobacteria; Dechloromonas	0.3	0.1	0	4.4	1.7	0.8	0.3	0	0	0
Alphaproteobacteria; MNG7	0.4	0.1	0.6	0.6	1.2	0.9	1.1	0.6	0.5	1.5
Actinobacteria; Fodinicola	0.5	0.1	0.3	0.5	1.1	1	1	0.6	0.3	1.2
Gammaproteobacteria; QEEB1BB10	0.1	0	0.1	0.7	1.2	1	1.2	0.4	0.4	1.1
Actinobacteria; f__PeM15_OTU_2935	1.8	0.3	1.6	0.2	0.1	0.2	0.2	0.4	1	0.3
Bacteroidetes; vadinBC27 wastewater−sludge group	5	0	0.1	0.2	0.4	0.1	0.1	0	0	0
Alphaproteobacteria; Rhodobacter	0.1	0.1	0.1	1	1.2	0.6	0.8	0.4	0.2	1.1
Bacteroidetes; MK04	0.1	0	0.1	0.9	1	1	1.3	0	0	1.2
Actinobacteria; B1−K2−141	0.2	0	0.2	0.4	0.7	0.7	1.1	0.8	0.3	1.1
Betaproteobacteria; Simplicispira	0	0	0	0.7	1.3	0.6	1.4	0.1	0	1.3
Actinobacteria; K2−78	0	0	0.1	0.3	0.7	0.7	1.4	0.6	0.2	1.1

3.4 Figure 2: Principal component analysis

Identification of samples with similar microbial communities using multivariate statistics (PCA). Each point represent 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.



4 Materials and methods

4.1 DNA extraction

4.1.1 FastDNA SPIN Kit for Soil

DNA extraction was performed using 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).

4.2 Bacterial community analysis targeting 16S V1-3 rRNA

4.2.1 Library preparation

Bacteria 16S V1-3 rRNA gene sequencing libraries were prepared by 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 the bacteria 16S V1-3 rRNA gene amplicons. Each PCR reaction (25 μL) contained dNTPs (100 μM of each), $MgSO_4$ (1.5 mM), Platinum Taq DNA polymerase HF (0.5 U/reaction), Platinum High Fidelity buffer (1X) (Thermo Fisher Scientific, USA) and barcoded library adaptors (400 nM of each forward and reverse). PCR was conducted with the following program: Initial denaturation at 95 °C for 2 min, 30 cycles of amplification (95 °C for 20 s, 56 °C for 30 s, 72 °C for 60 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 16S V1-3 specific primers: [27F] AGAGTTTGATCCTGGCTCAG and [534R] ATTACCGCGGCTGCTGG (Ward et al., 2012). The resulting amplicon libraries were purified using the standard protocol for Agencourt Ampure XP Beads (Beckman Coulter, USA) with a bead to sample ratio of 4:5. DNA was eluted in 25 μL of nuclease free water (Qiagen, Germany). DNA concentration was measured using Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Gel electrophoresis using Tapestation 2200 and D1000/High sensitivity D1000 screentapes (Agilent, USA) was used to validate product size and purity of a subset of sequencing libraries.

4.2.2 DNA sequencing

The purified sequencing libraries were pooled in equimolar concentrations and diluted to 6 nM. The samples were paired-end sequenced (2x300 bp) on a MiSeq (Illumina, USA) using a MiSeq Reagent kit v3 (Illumina, USA) following the standard guidelines for preparing and loading samples on the MiSeq. >10% PhiX control library was spiked in to overcome low complexity issues often observed with amplicon samples.

4.2.3 Bioinformatic processing

Forward and reverse reads were trimmed for quality using Trimmomatic v. 0.32 (Bolger et al., 2014) with the settings SLIDINGWINDOW:5:3 and MINLEN:275. The trimmed forward and reverse reads were merged using FLASH v. 1.2.7 (Magoč and Salzberg, 2011) with the settings -m 10 -M 200. The trimmed reads were dereplicated and formatted for use in the UPARSE workflow (Edgar, 2013). The dereplicated reads were clustered, using the usearch v. 7.0.1090 -cluster_otus command with default settings. OTU abundances were estimated using the usearch v. 7.0.1090 -usearch_global command with -id 0.97 -maxaccepts 0 -maxrejects 0. Taxonomy was assigned using the RDP classifier (Wang et al., 2007)

Genomics price example (standard package)

Technology	Assembly strategy	Assembly contiguity	Consensus accuracy	Turn-around-time*	List price/sample**
Illumina	Short-read	Typically fragmented	> 99.99 %	5 weeks	300 EUR
Oxford Nanopore	Long-read	Highly contiguous	> 99.9 %	2 weeks	250 EUR
Illumina + Oxford Nanopore	Hybrid	Highly contiguous	> 99.99 %	5 weeks	450 EUR

*Estimated turnaround time, (enquire for fast TAT) **Base of price estimate: minimum of 24 samples

Founded in 2014, DNASense's mission is to make cutting-edge DNA sequencing and bioinformatics readily available to life science research and industry