MIBIREM – Toolbox for Microbiome based Remediation

Deliverable 2.1

Lists of microbiome taxonomic diversity

Due Date:	30 November 2023
Submission Date:	30 November 2023
Dissemination Level:	PUBLIC
Lead Beneficiary:	UHAS
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Project Acronym: MIBIREM	**Project Number**: 101059260
Start Date of Project: 01/10/2022	**End Date of Project**: 31/03/2027

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Document Control Information	
Title	*Lists of microbiome taxonomic diversity*
Editor	*Sofie Thijs (UHAS)*
Contributors	*AIT, CNRS, UNIPI, UGENT*
Reviewer/s	*Thomas Reichenauer (AIT), Aurelie Cébron (CNRS)*
Dissemination Level (please choose just one according to the DoA)	☐ **SEN** - Sensitive (please provide one-page Publishable Summary) ☒ **PU** - Public
Approved by (please tick the boxes after all the partners have provided their written approval of the deliverable)	☒ RTDS (COO) ☒ AIT (SCO) ☒ UHAS ☒ CNRS ☒ UGENT ☒ SENSA ☒ ALTAR ☒ DND ☒ UU ☒ UNIPI ☒ Tauw
Underlying IPRs	*Are there intellectual property rights included in this deliverable? If yes, please specify.* **No**
Underlying Datasets	*Are there relevant datasets included in this deliverable? If yes, please specify.* **Yes, list of bacterial strains. Partial 16Sr RNA gene Sanger sequences in fasta format.**

Version/Date	***Change/Comment***
Version 1; 24/11/2023	*First draft version of UHAS*
Version 2; 30/11/2023	*Final version of UHAS, last edits included*

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1 Summary

This deliverable describes the identification of the microbial taxonomic diversity of all sampled sites by 16S rRNA gene amplicon sequencing metabarcoding to establish links between community structure and pollutant/metabolite concentrations and to infer functional traits of the different microbiomes (D2.1). Copy number of degradative strains and genes are determined with (q)PCR by primers for degradative genes (e.g. AlkB, PAH-RHD, Naphthalene dioxygenase). Novel and existing bioinformatics pipelines are used to streamline the prediction of biochemical pathways, using Picrust or Tax4fun.

Up to date, nine PHC sites have been sampled, 7 cyanide polluted sites and two HCH sites. The soil, groundwater, bactraps or biochar samples have been received by the labs (UHAS, CNRS, AIT, UNIPI, UGENT) to start DNA-extractions followed by library preparation and amplicon sequencing (V4; 515f-806r) to identify the total microbial diversity.

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2 Introduction

From all sampled sites (see cf. D1.1), soil and groundwater samples were provided for the isolation of DNA as well as to determine the environmental conditions these microbiomes live in.

2.1 Sample overview

The following samples were received and DNA-extracted according to the procedures below:

- **Griftpark Utrecht**
  - Soil core: SC1-SC6; each in triplicate
  - Liners:
    - MF1A_550423545
    - MF1A_550423564
    - MF3A_550409694
    - MF3A_550409695
    - MF01C_550409699
    - MF01C_550423558
    - MF01C_550409698
    - MF03B_550409697
    - MF02_550423424
    - MF02_550445749
    - MF02_550416977
    - MF02_550411525
    - MF02_550416976
    - MF02_550423509
- **Amersfoort (Vetgasfactory):**
  -

Sample Name	Depth (cm)
A044 18-10-2016	17,5-18
A044 18-10-2016	19,5-20
A044 18-10-2016	21,5-22
A044 18-10-2016	23,5-24
A044 18-10-2016	25,5-26
A044 18-10-2016	27,5-28
A044 18-10-2016	29,5-30
A044 18-10-2016	33,5-34
A044 18-10-2016	35,5-36
A044 18-10-2016	37,5-38
A044 18-10-2016	39,5-40
A044 18-10-2016	41,5-42
A044 18-10-2016	43,5-44
A044 18-10-2016	45,5-46
A044 18-10-2016	53,5-54

- **Tauw Lumco site in Gent:**

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Code	reservoir type
BE_GEN_01_W	1L bottle groundwater
BE_GEN_02_W	1L bottle groundwater
BE_GEN_03_W	1L bottle groundwater
BE_GEN_05_W	1L bottle groundwater
BE_GEN_06_W	1L bottle groundwater
BE_GEN_07_W	1L bottle groundwater
BE_GEN_01_W	1L bottle groundwater
BE_GEN_02_W	1L bottle groundwater
BE_GEN_03_W	1L bottle groundwater
BE_GEN_05_W	1L bottle groundwater
BE_GEN_06_W	1L bottle groundwater
BE_GEN_07_W	1L bottle groundwater
BE_GEN_01_B_S	soil
BE_GEN_02_B_S	soil
BE_GEN_01_B_S	soil
BE_GEN_02_B_S	soil
BE_GEN_01_B_S	soil
BE_GEN_02_B_S	soil

- **Tauw-Kralingen**

Sample Name	Code
1384296	5059
1384296	5062
1384296	5101
1384296	5104
1384296	5107
1384296	5111
Effluent	Effluent
1384296	5059_groot
1384296	5062_groot
1384296	5101_groot
1384296	5104_groot
1384296	5107_groot
1384296	5111_groot
Effluent	Effluent_groot
1384296	5059_klein
1384296	5062_klein
1384296	5101_klein
1384296	5104_klein
1384296	5107_klein
1384296	5111_klein
Effluent	Effluent_klein

- **CNRS:**

PLO_01_A_S
PLO_01_B_S
PLO_02_A_S
PLO_02_B_S
PLO_03_A_S
PLO_03_B_S
PLO_04_A_S
PLO_04_B_S
PLO_05_A_S
PLO_05_A_S
PLO_01_W
PLO_02_W
PLO_03_W
PLO_04_W

- **UNIPI:**

  Bitterfeld, Germany

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sample
DE_BIT_01_A_S
DE_BIT_02_A_S
DE_BIT_03_A_S
DE_BIT_04_A_S
DE_BIT_05_A_S
DE_BIT_06_A_S
DE_BIT_07A_S
DE_BIT_08_A_S
DE_BIT_09_A_S
DE_BIT_10_A_S

Colleferro, Italy:

sample
IT_COL_01
IT_COL_02

- **Siebenhirten, Austria**

AT_SIE_01A_GW; AT_SIE_01B_GW; AT_SIE_02A_GW; AT_SIE_02B_GW; AT_SIE_03_GW; AT_SIE_01A_Btr; AT_SIE_01B_Btr; AT_SIE_02A_Btr; AT_SIE_02B_Btr; AT_SIE_03_Btr;

- **Simmering, Austria**

AT_SIM_01A_GW; AT_SIM_01B_GW; AT_SIM_02A_GW; AT_SIM_02B_GW; AT_SIM_03_GW; AT_SIM_01A_Btr; AT_SIM_01B_Btr; AT_SIM_02A_Btr; AT_SIM_02B_Btr; AT_SIM_03_Btr;

- **Rinteln, Germany**

DE_RIN_01_GW; DE_RIN_02_GW; DE_RIN_03_GW; DE_RIN_04_GW; DE_RIN_05_GW; DE_RIN_06_GW; DE_RIN_07_GW; DE_RIN_08_GW; DE_RIN_09_GW; DE_RIN_10_GW; DE_RIN_01_Btr; DE_RIN_02_Btr; DE_RIN_03_Btr; DE_RIN_04_Btr; DE_RIN_05_Btr; DE_RIN_01_A_S; DE_RIN_01_B_S; DE_RIN_01_C_S; DE_RIN_01_D_S; DE_RIN_02_A_S; DE_RIN_02_B_S; DE_RIN_02_C_S; DE_RIN_02_D_S; DE_RIN_02_E_S; DE_RIN_03_A_S; DE_RIN_03_B_S; DE_RIN_03_C_S; DE_RIN_03_D_S

- **Stockach, Germany**

DE_ST_01_Btr; DE_ST_02_Btr; DE_ST_03_Btr; DE_ST_04_Btr; DE_ST_05_Btr; DE_ST_01_GW; DE_ST_02_GW; DE_ST_03_GW; DE_ST_04_GW; DE_ST_05_GW

- **Rotterdam, The Netherlands**

7025A; 7025B; 7026A; 7026B; 7009

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2.1.1 DNA extraction procedures

The consortium partners agreed on the following DNA-extractions protocols, and all soil and groundwater samples were processed in the same way.

2.1.1.1 DNA extraction from groundwater

- Take 2x1L of Groundwater samples (use autoclaved glass bottles and rinse them with the pumped water before filling them up)
- Transport samples to the lab cooled at 4°C
- Keep samples at 4°C until filtering
- Filter bacteria using a 0.45µm filter (same day, or next day, preferably not later).
    - MicroFunnel 300 with GN-6 Filter 0.45 µm (Pall Corporation).
    - Depending on the fine material you might have in the groundwater samples, filtering might take a long time, thus I suggest to first take all the samples and then start with filtering.
    - If you do just the filtering at the site, you have to freeze the filter immediately at -20°C for the transport to the lab.
- Immediately after filtration, freeze and store the filter at -20°C
- Extract DNA with DNeasy Power Water Kit (DNeasy PowerWater Kit (qiagen.com))
- Store DNA at -20°C

2.1.1.2 DNA extraction from soil

- Collect soil samples in either zip lock bags or glass pots (amount, minimum 250 gram, to homogenise well). If sampling from a certain layer, e.g. 50-100 cm, then sometimes less soil is collected, in liners. This is also fine, because you might be interested in the niche-specific bacterial communities (aerobic, anaerobic, rooting zone, humus or clay layer, groundwater fringe layer…).
- Make sure to clean soil sampling material between sampling spots (e.g. dWater, deconex, ETOH)
- Transfer soil samples to the lab cooled at 4°C
- Sieve the soil to 2mm (if not possible use a bigger mesh and record)
- Store aliquot of soil (*e.g.* 5g) at -20°C
- Use another fraction of the soil to determine water content (measure fresh soil weigh and dry soil weight after dry at 60°C) for calculation of the amount of DNA per dry weight of soil
- Use FastPrep (FastPrep-24™ Classic Instrument | MP Biomedicals) to grind soil sample: (parameters (duration and intensity) can be adapted, in case of different soil types instead use manufacturer

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recommendation; tubes should be filled up to two third of the volume (more soil volume might reduce DNA yield, record the amount of soil (weight) used for the gDNA extraction)

- Use MP Kit (FastDNA™ Spin Kit for Soil DNA Extraction | MP Biomedicals) for extraction of DNA
- Store DNA at -20°C

All DNA samples were checked on gel, concentration determined with qubit, and purity (260/280; 230/260) with the nanodrop.

2.1.2 Library preparation and sequencing

Individual pathways were used for the three different use-cases.

DNA was sent directly to Novogene to perform the complete library prep followed by partial 16S rRNA gene sequencing on the Noveseq (2 x 250 bp). In total 150 samples were sent to Novogene for sequencing with 30K reads per sample as the desired outcome.

No	Part Number	Product Name	Description
1	RSMD00212	Amplicon Metagenomics Sequencing (WBI)	PCR amplification
2			DNA sample QC
3			Amplicon library preparation
4			Illumina Sequencing PE250 (30 K tags per sample)
5			Standard Analysis-QIIME 2
6			Cloud Delivery

The kit used for library preparation is: NEBNext® Ultra™ IIDNA Library Prep Kit (Cat No. E7645). PCR amplification of targeted regions is performed by using specific primers connecting with barcodes. Sequences of primers used:

515F-improved: GTGYCAGCMGCCGCGGTAA

806R-improved: GGACTACNVGGGTWTCTAAT

Generating an amplicon of the V4-region of 291 bp. The PCR products with proper size are selected by 2% agarose gel electrophoresis. Same amounts of PCR products from each sample are pooled, end-repaired, A-tailed and further ligated with Illumina adapters. Libraries are sequenced on a paired-end Illumina platform to generate 250bp paired-end raw reads. The experimental procedures of DNA library preparation are shown in Figure 1.

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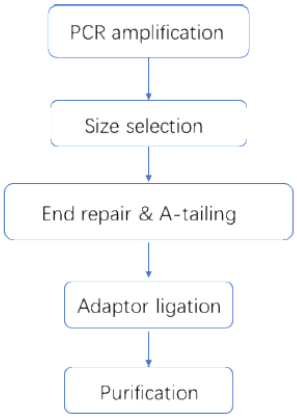

Figure 1 Workflow of library construction

The library is checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. Quantified libraries are pooled and sequenced on Illumina platforms, according to effective library concentration and data amount required. We require 30ktags and most of the time we are at saturation. The platform used is NovaSeq6000,

2.1.2.1 Library QC

All passed the quality control step.

2.1.3 Results of 16S rRNA gene amplicon sequencing

At the moment, samples are being sequenced and awaiting the next bioinformatics data processing.

UNIPI soil samples were sequenced and taxonomically analysed.

- ASV table
- REP.SEQ
- TAXONOMY
- METADATA

Primers utilized for metabarcoding:

515F GTGCCAGCMGCCGCGGTAA

907R CCGTCAATTCCTTTGAGTTT

Different sets of primers were designed for the qPCR on the LinA gene (first dehalogenase enzyme of the process of HCH degradation).

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LinA primers were designed by using PrimerBlast using all complete CDS reports recovered in NCBI.

A set of primers (the number 7) was recovered from the literature.

Primers will be tested on the enriched microbiota.

Group	F	R	Source
1	TTGGTGGGATGATGCAGAG	CGCGCTCACAAATTCCA	Primer Blast
2	GGGATGATGCAGAGTGGA	GTCCGCGCTCACAAATTC	Primer Blast
3	TTGGTGGGATGATGCAGA	TCCGCGCTCACAAATTC	Primer Blast
4	GGTGGGATGATGCAGAGT	CCGCGCTCACAAATTCC	Primer Blast
5	TGGGATGATGCAGAGTGG	TGTCCGCGCTCACAAAT	Primer Blast
6	TTGGTGGGATGATGCAGAG	TCCGCGCTCACAAATTCC	Primer Blast
7	GCGGATCCGCCATGAGTGATCTAGAGACAGACTT	GCCTCGAGTTATGCGCCGGACGGTGCGAAATG	10.1002/jobm.201300211

3 Annex

See on the next page the sequenced samples at NOVOGENE.

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Sample Name (Required)	Sample Type (Required)	Tissue/Nucleic Acid Type (Required)	Nucleic Acid Type Desc	Species Type (Required)	Species Latin Name (Required)	Tissue/Organ (Required)	Sample Status (Required)	Other Sample Status Notes	Concentration (ng/ul)	Volume(ul)	PCR Products Fragment Size	Amplification region (Required): please use the updated 515f-806r primers	2nd Amplification region (Required)
SV1	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		43.4	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV2	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		16.4	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV3	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		53.7	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV4	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		33.4	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV5	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		31.3	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV6	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		34.6	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV7	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		27.1	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV8	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		125.2	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV9	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		33.3	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV10	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		137.8	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV11	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		21.9	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV12	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.7	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV13	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		113.9	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV14	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		60.6	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV15	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		38.7	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV16	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		61.3	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV17	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		71.5	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV18	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		20.8	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV19	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		14	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV20	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		21.8	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV21	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		25.4	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV22	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		19.5	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV23	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		43.4	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV24	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		31.8	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV25	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		83.4	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV26	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		84.6	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV27	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		139	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV28	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		40.6	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV29	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		30.6	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV30	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		12.7	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV31	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		42.8	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV32	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		33.5	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV33	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.223	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV34	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.252	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV35	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.239	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV36	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.336	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV37	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.348	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV38	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.176	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV39	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.105	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV40	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.124	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV41	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.17	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV42	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.21	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV43	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.122	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV44	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.115	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV45	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.102	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV46	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.134	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV47	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.14	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV48	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.242	50		Bacterial 16S V4	
SV49	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.185	50		Bacterial 16S V4	
SV50	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.221	50		Bacterial 16S V4	
SV51	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.196	50		Bacterial 16S V4	
SV52	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.206	50		Bacterial 16S V4	
SV53	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.385	50		Bacterial 16S V4	
SV54	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.225	50		Bacterial 16S V4	
SV55	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.264	50		Bacterial 16S V4	
SV56	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.4	50		Bacterial 16S V4	
SV57	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.164	50		Bacterial 16S V4	
SV58	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.216	50		Bacterial 16S V4	
SV59	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.114	50		Bacterial 16S V4	
SV60	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.135	50		Bacterial 16S V4	
SV61	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.524	50		Bacterial 16S V4	
SV62	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.218	50		Bacterial 16S V4	

Sample Name (Required)	Sample Type (Required)	Tissue/Nucleic Acid Type (Required)	Nucleic Acid Type Desc	Species Type (Required)	Species Latin Name (Required)	Tissue/Organ (Required)	Sample Status (Required)	Other Sample Status Notes	Concentration (ng/ul)	Volume(ul)	PCR Products Fragment Size	Amplification region (Required): please use the updated 515f-806r primers	2nd Amplification region (Required)
SV63	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.32	50		Bacterial 16S V4	
SV64	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.189	50		Bacterial 16S V4	
SV65	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.257	50		Bacterial 16S V4	
SV66	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.17	50		Bacterial 16S V4	
SV67	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.09	50		Bacterial 16S V4	
SV68	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.118	50		Bacterial 16S V4	
SV69	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.1	50		Bacterial 16S V4	
SV70	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.305	50		Bacterial 16S V4	
SV71	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.676	50		Bacterial 16S V4	
SV72	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.424	50		Bacterial 16S V4	
SV73	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.128	50		Bacterial 16S V4	
SV74	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.219	50		Bacterial 16S V4	
SV75	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.175	50		Bacterial 16S V4	
SV76	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.468	50		Bacterial 16S V4	
SV77	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.18	50		Bacterial 16S V4	
SV78	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.322	50		Bacterial 16S V4	
SV79	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.258	50		Bacterial 16S V4	
SV80	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.632	50		Bacterial 16S V4	
SV81	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.556	50		Bacterial 16S V4	
SV82	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.13	50		Bacterial 16S V4	
SV83	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.19	50		Bacterial 16S V4	
SV84	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.121	50		Bacterial 16S V4	
SV85	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.21	50		Bacterial 16S V4	
SV86	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.134	50		Bacterial 16S V4	
SV87	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.177	50		Bacterial 16S V4	
SV88	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.66	50		Bacterial 16S V4	
SV89	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.186	50		Bacterial 16S V4	
SV90	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.134	50		Bacterial 16S V4	
SV91	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.26	50		Bacterial 16S V4	
SV92	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		3.24	50		Bacterial 16S V4	
SV93	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.22	50		Bacterial 16S V4	
SV94	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.876	50		Bacterial 16S V4	
SV95	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		11.4	50		Bacterial 16S V4	
SV96	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		4.32	50		Bacterial 16S V4	
SV97	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		7.12	50		Bacterial 16S V4	
SV98	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		7.4	50		Bacterial 16S V4	
SV99	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		5.64	50		Bacterial 16S V4	
SV100	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		3.5	50		Bacterial 16S V4	
SV101	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		17	50		Bacterial 16S V4	
SV102	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		2.54	50		Bacterial 16S V4	
SV103	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		2.37	50		Bacterial 16S V4	
SV104	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		79.2	50		Bacterial 16S V4	
SV105	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		4.2	50		Bacterial 16S V4	
SV106	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.82	50		Bacterial 16S V4	
SV107	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		1.9	50		Bacterial 16S V4	
SV108	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.332	50		Bacterial 16S V4	
SV109	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.52	50		Bacterial 16S V4	
SV110	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		87.2	50		Bacterial 16S V4	
SV111	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		1.08	50		Bacterial 16S V4	
SV112	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		1.73	50		Bacterial 16S V4	
SV113	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		4	50		Bacterial 16S V4	
SV114	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.844	50		Bacterial 16S V4	
SV115	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		1.93	50		Bacterial 16S V4	
SV116	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		2.42	50		Bacterial 16S V4	
SV117	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		4.68	50		Bacterial 16S V4	
SV118	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		2.26	50		Bacterial 16S V4	
SV119	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.527	50		Bacterial 16S V4	
SV120	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.323	50		Bacterial 16S V4	
SV121	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.259	50		Bacterial 16S V4	
SV122	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.02	50		Bacterial 16S V4	
SV123	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.12	50		Bacterial 16S V4	
SV124	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.296	50		Bacterial 16S V4	

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A	B	C	D	E	F	G	H	I	J	K	L	M	N
Sample Name (Required)	Sample Type (Required)	Tissue/Nucleic Acid Type (Required)	Nucleic Acid Type Desc	Species Type (Required)	Species Latin Name (Required)	Tissue/Organ (Required)	Sample Status (Required)	Other Sample Status Notes	Concentration (ng/ul)	Volume(ul)	PCR Products Fragment Size	Amplification region (Required): please use the updated 515f-806r primers	2nd Amplification region (Required)
SV125	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.087	50		Bacterial 16S V4	
SV126	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.649	50		Bacterial 16S V4	
SV127	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.537	50		Bacterial 16S V4	
SV128	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		1.04	50		Bacterial 16S V4	
SV129	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.358	50		Bacterial 16S V4	
SV130	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.294	50		Bacterial 16S V4	
SV131	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		9.88	50		Bacterial 16S V4	
SV132	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		7.88	50		Bacterial 16S V4	
SV133	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		4.88	50		Bacterial 16S V4	
SV134	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		14.8	50		Bacterial 16S V4	
SV135	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		13.9	50		Bacterial 16S V4	
SV136	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.456	50		Bacterial 16S V4	
SV137	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		1.12	50		Bacterial 16S V4	
SV138	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.784	50		Bacterial 16S V4	
SV139	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		3.7	50		Bacterial 16S V4	
SV140	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		1.41	50		Bacterial 16S V4	
SV141	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.732	50		Bacterial 16S V4	
SV142	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		0.432	50		Bacterial 16S V4	
SV143	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		34.2	50		Bacterial 16S V4	
SV144	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		8.56	50		Bacterial 16S V4	
SV145	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		2.67	50		Bacterial 16S V4	
SV146	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		112	50		Bacterial 16S V4	
SV147	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		108.6	50		Bacterial 16S V4	
SV148	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		162	50		Bacterial 16S V4	
SV149	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		146	50		Bacterial 16S V4	
SV150	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		86	50		Bacterial 16S V4	
SV151	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		94	50		Bacterial 16S V4	
SV152	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		107	50		Bacterial 16S V4	
SV153	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		76	50		Bacterial 16S V4	
SV154	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		53.8	50		Bacterial 16S V4	
SV155	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		215.5	50		Bacterial 16S V4	
SV156	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		145.3	50		Bacterial 16S V4	
SV157	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		56.6	50		Bacterial 16S V4	
SV158	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		117	50		Bacterial 16S V4	
SV159	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		86	50		Bacterial 16S V4	
SV160	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		100	50		Bacterial 16S V4	
SV161	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		90.4	50		Bacterial 16S V4	
SV162	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		168	50		Bacterial 16S V4	
SV163	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		178	50		Bacterial 16S V4	
SV164	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		112	50		Bacterial 16S V4	
SV165	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		116	50		Bacterial 16S V4	
SV166	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		87	50		Bacterial 16S V4	
SV167	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		78	50		Bacterial 16S V4	
SV168	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		61.6	50		Bacterial 16S V4	
SV169	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		156.3	50		Bacterial 16S V4	
SV170	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		29.4	50		Bacterial 16S V4	
SV171	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		88.2	50		Bacterial 16S V4	
SV172	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		111	50		Bacterial 16S V4	
SV173	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		10.8	50		Bacterial 16S V4	
SV174	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		108	50		Bacterial 16S V4	
SV175	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		105.4	50		Bacterial 16S V4	
SV176	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		138	50		Bacterial 16S V4	
SV177	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		221	50		Bacterial 16S V4	
SV178	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		96	50		Bacterial 16S V4	
SV179	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		110	50		Bacterial 16S V4	
SV180	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		83	50		Bacterial 16S V4	
SV181	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		192	50		Bacterial 16S V4	
SV182	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		123.4	50		Bacterial 16S V4	
SV183	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		130.4	50		Bacterial 16S V4	
SV184	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		43.6	50		Bacterial 16S V4	
SV185	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		56.6	50		Bacterial 16S V4	

A	B	C	D	E	F	G	H	I	J	K	L	M	N
Sample Name (Required)	Sample Type (Required)	Tissue/Nucleic Acid Type (Required)	Nucleic Acid Type Desc	Species Type (Required)	Species Latin Name (Required)	Tissue/Organ (Required)	Sample Status (Required)	Other Sample Status Notes	Concentration (ng/ul)	Volume(ul)	PCR Products Fragment Size	Amplification region (Required): please use the updated 515f-806r primers	2nd Amplification region (Required)
SV187	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer		55.3	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV188	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved in ddH2O		0.0316	50		Bacterial 16S V4	Bacterial 16S V4-V5
SV189	DNA	Genomic DNA		bacteria	metagenome	soil	Dissolved TE Buffer						

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