Marine

Microbial Biodiversity,

Bioinformatics & Biotechnology

Ocean Sampling Day Handbook

Version of April 2016

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Summary

The Ocean Sampling Day (OSD) Handbook, version of April 2016, is a best practice guide describing procedures and policies on the marine sample collection, logistics and bioinformatics intended primarily for marine research stations and cruises contributing to the Ocean Sampling Day event, (http://www.microb3.eu/osd). These guidelines on the OSD sample and data collection and archiving were developed by the Micro B3 consortium to support an objective of the Micro B3 project (http://www.microb3.eu/) to integrate global marine data with research on microbial diversity and functions.

A collection of OSD samples acquired according to OSD Handbook guidelines, i.e. using standardised protocols and accompanied by a standardised set of environmental parameters, will enable molecular and morphological analysis of marine microbial biodiversity on a global scale and in a rich environmental context. OSD samples will be archived at the Smithsonian Institution National Museum of Natural History, USA, to allow their availability as technologies advances. OSD sample metadata and environmental data will be stored at the PANGAEA (http://www.pangaea.de), condensed summary of oceanographic data in the SeaDataNet (http://www.seadatanet.org/) and morphology-based biodiversity data in the EurOBIS (http://www.eurobis.org/). OSD sample metadata and sequence/read data will be archived at the ENA (http://www.ebi.ac.uk/ena/). The Micro B3 Information System will provide a primary access to all OSD data.

List of abbreviations

ABS	Access Benefits Sharing
BAS	British Antarctic Survey, UK
CBD	Convention on Biological Diversity
CIESM	The Mediterranean Science Commission, Monaco
CNRS	Centre National de la Recherche Scientifique, France
CSIC	agencia estatal Consejo Superior de Investigaciones Científicas, Spain
EBI	European Bioinformatics Institute, UK
EMBL	European Molecular Biology Laboratory
EMPA	Environmental & Marine Project Management Agency, Germany
ENA	European Nucleotide Archive at the EMBL-EBI, UK
EurOBIS	European Ocean Biogeographic Information System, Belgium
HCMR	Hellenic Centre for Marine Research, Greece
JacobsUni	Jacobs University Bremen gGmbH, Germany
MBA	Marine Biological Association of the United Kingdom, UK
MARIS	Marine Information Services, The Netherlands
MAT	Mutually Agreed Terms
Micro B3	Biodiversity, Bioinformatics, Biotechnology
Micro B3 IS	Micro B3 Information System
MPIMM	Max Planck Institute for Marine Microbiology, Germany
MTA	Material Transfer Agreement
NODC	National Oceanographic Data Centre
OSD	Ocean Sampling Day
PANGAEA	Data Publisher for Earth and Environmental Science, Germany
PIC	Prior Informed Consent
SeaDataNet	Pan-European Infrastructure for ocean & marine data management
SI NMNH	Smithsonian Institution National Museum of Natural History, USA
UniHB	University of Bremen, Germany
UOXF	University of Oxford, UK
VLIZ	Vlaams Instituut voor de Zee, Belgium

Purpose of the Ocean Sampling Day Handbook

The Micro B3 (Biodiversity, Bioinformatics, Biotechnology) project (2012-2015) http://www.microb3.eu/, was a highly interdisciplinary project intending to develop a standardised best sampling practice, legal framework and bioinformatics technology for marine sample data collection, archiving and analysis, with the objective to integrate global marine data with research on microbial diversity and functions.

The Ocean Sampling Day (OSD) Handbook is a best practice guide describing procedures and policies on the marine sample collection, logistics and bioinformatics developed by the Micro B3 Consortium primarily for marine research stations and cruises contributing to the sampling event on the Ocean Sampling Day.

The Ocean Sampling Day, http://www.microb3.eu/osd, is a simultaneous sampling campaign of the world's oceans taking place for the third time on the solstice (21st June) in the year 2016.
This massive sampling activity of marine sites and groups around the world, both scientific and non-scientific, will create a collection of OSD samples acquired in a standardised way and accompanied by a standardised set of environmental parameters. The OSD samples will then be available for nucleotide sequence analysis. Obtained marine molecular data integrated with a rich environmental context will allow modelling of marine ecosystems and shall provide a better insight into the role that marine microbial complexity plays in climate changes.
The Ocean Sampling Day has been an excellent use case for development of Micro B3 infrastructures that will now support not only the Ocean Sampling Day 2016 but also other future marine sampling enterprises.

In addition to providing best practice on the collection of marine samples and data, this Handbook provides users with essential guidelines on how to deposit acquired Micro B3/OSD data and how these data will be archived and shared.

Disclaimer:
This Ocean Sampling Day Handbook, version of April 2016, is a best practice guide at the time of release of the Handbook. It reflects sampling procedures, policies, development of the Micro B3 Information System as well as integration level of involved data archives at the time of the Handbook release.

## Content of the Ocean Sampling Day Handbook

1. Ocean Sampling Day Workflow

An OSD sampling Site is throughout this Handbook defined as a marine research station or cruise participating in the Ocean Sampling Day 2016. Each OSD sampling Site shall follow the Micro B3 best practice workflow shown schematically in the **Figure 1** and described in the steps below:

1. OSD sampling Site registers for the OSD (please see the Chapter *OSD Site Registration*).

2. OSD sampling Site coordinator signs the OSD Data Sharing Policy (please see the Chapter *OSD Data Sharing Policy* and the *Annex V*).

3. OSD sampling Site obtains required sampling permissions before the OSD sampling begins (please see the Chapter *OSD Legal Permissions for Sampling* and *the Annex II and IV).*

4. OSD sampling Site downloads OSD Logsheets with the *OSD checklist* of sample contextual data (please see the *Annex I).*

5. **OSD sampling**. OSD sampling Site uses for the OSD sampling standardised protocols and instructions (please see the Chapter *OSD Sampling Instructions & Protocols* and *OSD Mandatory and Recommended Information*)

6. OSD sampling Site dispatches a minimum of four to six OSD samples to members of the OSD Core Team in the Max Planck Institute for Marine Microbiology in Bremen, Germany. The package will also contain (1) a copy of the OSD Logsheets with the completed *OSD checklist* and (2) relevant documents (please see the Chapter *Handling OSD Samples (shipping, sequencing and bio-archiving)).*

7. OSD sampling Site is encouraged to store additional biological replicates of the OSD samples locally according to storage instructions of the OSD sampling protocols.

8. OSD sampling Site submits OSD sample contextual data of the *OSD checklist to* the Micro B3 Information System (please see the Chapter *Submitting OSD Sample Contextual Data).*

9. OSD sampling sites is contacted by the PANGAEA Team who will advise on submission of environmental and morphology-based biodiversity data (please see the Chapter *OSD Environmental and Morphology-based Biodiversity Data*)

10. OSD sampling Site can access nucleotide sequence data from the OSD samples at the ENA (please see the Chapter *OSD Nucleotide Sequence Data)*

11. OSD sampling Site can access data analysis of OSD samples at the Micro B3 Information System, which will also provide integrated view of all data acquired during the OSD (please see the Chapter *OSD Data Access and Analysis*).
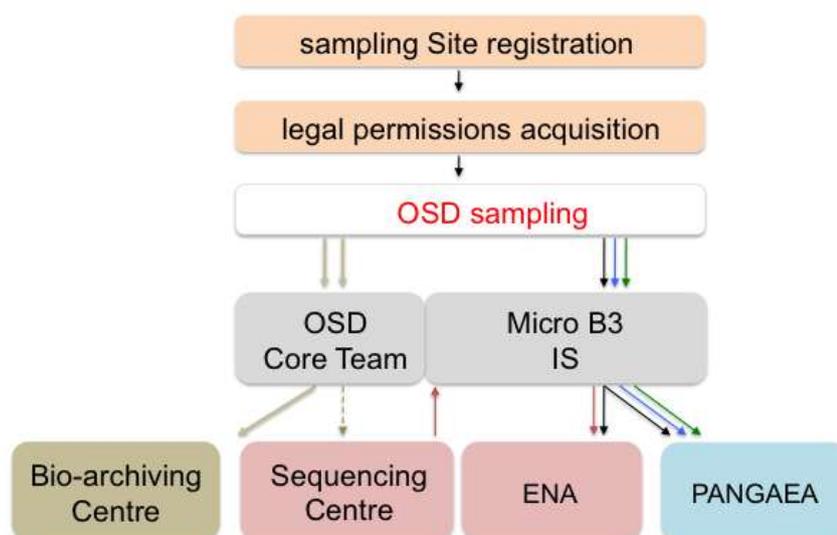
Figure 1: The Micro B3 best practice workflow for OSD Sampling Sites.
Sampling stations and cruises will need to register for the OSD and obtain required legal
sampling permissions before the OSD sampling begins. OSD samples shall be collected
according to Micro B3-standardised protocols and sampling instructions. Each OSD sample
shall be accompanied by at minimum *mandatory OSD checklist* metadata and as many as
possible Micro B3-recommended environmental data measurements. The OSD Core Team
will collect OSD samples, perform DNA extraction from these samples and send it for
nucleotide sequence analysis. A replicate of each sample will be cryo-preserved at the SI
NMNH. The mandatory OSD checklist information shall be submitted to the Micro B3 IS via
the Micro B3 IS submission portal.
Legend: brown full line – material transfer, brown dashed line – DNA transfer, **black full
line** – metadata transfer, blue full line – oceanographic data transfer, green full line –
biodiversity data transfer, red full line – molecular data transfer.

Micro B3 will collate environmental, morphological and molecular data originated from the
Micro B3 OSD sample collection and contextual data acquisition. Information recorded by
sampling groups during the OSD will be shared among environmental, biodiversity and
nucleotide sequence data archives.
The flow of samples and data is depicted in the **Figure 2**. OSD sampling Sites will submit
electronically content of the *OSD Logsheets* (containing contextual data of each OSD
sample, please see Chapter 6) to the Micro B3 IS. A paper copy of the *OSD Logsheets* will
accompany OSD samples, which shall be shipped to members of the OSD Core Team in
MPIMM, Germany. The OSD Core Team will collate OSD samples, extract DNA according to
standardised protocol and send it for a nucleotide sequence analysis to a sequencing
company. Molecular data will then be sent back to the Micro B3 IS, who will deposit the

mandatory OSD checklist metadata and sequence data from all OSD samples to the ENA (European Nucleotide Archive at the European Molecular Biology Laboratory, European Bioinformatics Institute, http://www.ebi.ac.uk/ena/). The PANGAEA (Data Publisher for Earth and Environmental Science; http://www.pangaea.de) will receive from the Micro B3 IS the *mandatory OSD checklist* and will contact after the OSD sampling event all OSD Sites and advise on how to submit OSD environmental and morphological data. Condensed summary of the sampling event at each OSD Site will flow into the SeaDataNet information system (http://www.seadatanet.org/), whereas biodiversity data based on morphology will flow to the EurOBIS (The European Ocean Biogeographic Information System, http://www.eurobis.org/). These three information systems (SeaDataNet, ENA and EurOBIS) will be connected to the Micro B3 IS, which will provide integration and a primary access to all OSD data as well as access to OSD data analysis workflows.
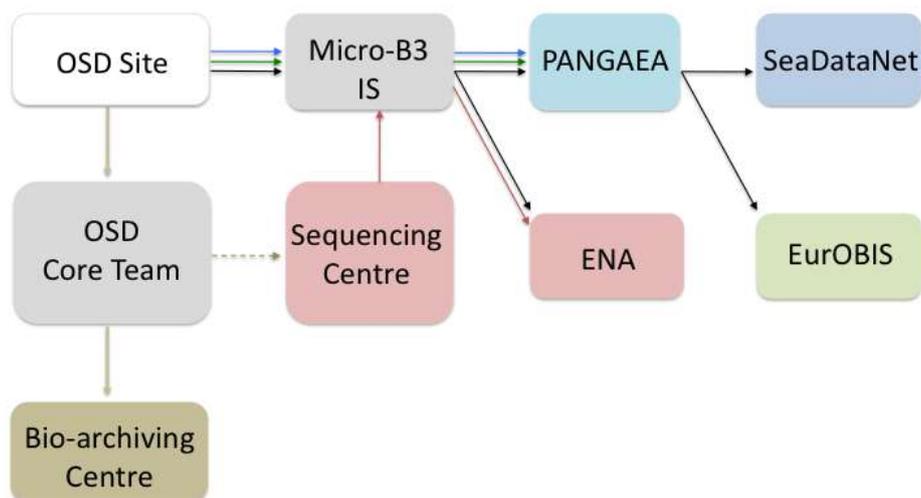
Figure 2: **The Micro B3 flow of OSD samples and data.**
OSD Sites will send their sampled material to the OSD Core Team, who will extract DNA from each sample and send it to the sequencing centre. A replicate of each sample will be archived at the SI NMNH. Sample *mandatory OSD checklist* metadata will be collected via the Micro B3 IS submission portal. The Micro B3 IS will then distribute relevant metadata to both ENA and PANGAEA. Oceanographic and biodiversity data will be archived at the PANGAEA, who will propagate a condensed summary of each OSD sampling event to both SeaDataNet and EurOBIS. Molecular data, obtained from sequencing company, will be archived at the ENA.
**Legend**: **brown full line** – material transfer, **brown dashed line** – DNA transfer, **black full line** – metadata transfer, **blue full line** – oceanographic data transfer, **green full line** – biodiversity data transfer, **red full line** – molecular data transfer.

2. OSD Site Registration

The OSD Consortium led an open call for participation in the Ocean Sampling Day to be held on the Solstice of June 21st, 2016. Scientists of marine research cruises and stations as well as non-scientists could get involved in the OSD, http://www.microb3.eu/osd. Interested parties could join OSD by formally expressing interest at the OSD website followed by an informative e-conference with an appointed sampling Site coordinator. Sites that provided minimum required information have been formally registered for participation at the OSD on a voluntary basis. An increasing number of Genomic Observatories form part of this growing OSD sampling Sites network. As of this date, 150+ sampling Sites registered for the OSD.

The OSD team in collaboration with the Micro B3 IS has built the *OSD Sites Registry,* a catalogue of marine sampling Sites involved in the OSD. The *OSD Sites* Registry will integrate a uniform description of the OSD Sites with environmental and sequence data following the 2016 OSD event and will be maintained and publicly available via the Micro B3 IS.

Sites in the *OSD Sites Registry* actively contributing to the OSD are formally considered members of the OSD Consortium. Samples of the OSD event on the Solstice of June 21st in the year 2015 will be bio-archived at the SI NMNH. OSD Sites will also have an access to OSD data analysis workflows available via the Micro B3 IS.

The *OSD Sites Registry* can be found at
http://mb3is.megx.net/osd-registry

Please contact the OSD team at osd-contact@microb3.eu for more information on registration for future OSD events.

3. OSD Data Sharing Policy

The OSD data sharing policy is a non-binding document aiming at harmonizing the management of the data that is produced from the analysis of the samples collected through the OSD.
The OSD data sharing policy draws on needs of the Micro B3 and OSD Community, on existing policies (Biosharing, http://biosharing.org/policies) and on best practice according to the *12 Step Path to a Data Policy* (Field *et al.*, 2009).

The **OSD data sharing policy** is generated by- and **applies to the members of the OSD Consortium**, i.e. those who are registered and substantively contribute to the OSD 2016 event.

The OSD data sharing policy is based on the following principles:

- Submission: Data will be submitted to the INSDC (ENA) and PANGAEA.
- Release: All data should be released as soon as sequenced to the public at large, i.e. to the public beyond the Micro B3/OSD Consortium, but respecting Ft. Lauderdale principles (http://www.genome.gov/pages/research/wellcomereport0303.pdf). Large sequencing projects routinely use the Ft. Lauderdale principles to promote public use of data while safeguarding contributions of the data generators. These principles entitle the data producers to make the first presentation and publish the first global analysis of the data.
- Access: The OSD dataset will be a reference dataset and should be as widely accessible and used to support downstream research as possible.
- Use: Once the OSD reference dataset is published OSD data can be freely used.
- Analysis: OSD Consortium encourages those interested in OSD data analysis to declare their interests and formally join the OSD Data Analysis Consortium, which is led by the OSD Core Team.
- Publications: It is expected that authorship of the global analysis of the OSD dataset to belong to the OSD Consortium. For specialist publications authorship will be defined on case-by-case basis.

Groups involved in the OSD, or anyone submitting or using data of the OSD Consortium should be aware of the OSD Data Sharing Policy, which is available in the **Annex V**.

4. OSD Legal Permissions for Sampling

The Micro B3 project is the framework within which the Ocean Sampling Day Initiative was initiated. The legal work undertaken by Micro B3 aimed at guaranteeing compliance with the international and national biodiversity law and law of the sea.

Marine scientific research activities need to be organized in full respect of national and international legal commitments in force in the states involved. The guidelines and model agreements below illustrate the steps to be followed within the Micro B3 project and ongoing OSD activities, but they can inspire other projects as well. These steps guarantee that legal certainty is achieved: this is beneficial for the research community, the provider countries and also the possible private investors.

Section 4.1 illustrates generally the steps to follow in order to identify what kind of permits are necessary in the country where a sampling is planned, and how to obtain them.

Section 4.2 illustrates Micro B3 ABS Model Agreement developed within the Micro B3 project. Its use is strongly recommended when sampling in countries where an ABS legislation is in place prescribing for Prior Informed Consent to be obtained. However, the Model Agreement can be used also as a negotiation base with countries that do not have ABS legislations in place. By using the public domain approach of the Micro B3 ABS Model Agreement (see 4.2) full compliance with international laws, with the OSD Data Policy and with the ethos of the Micro B3 project are guaranteed.

4.1. Legal Workflow for OSD participants regarding Permitting Requirements

The **Figure 3** summarizes **the legal workflow for OSD participants** regarding the legal steps to be undertaken in accessing the genetic resources and in transferring material and data to third parties, bioarchiving institutions or sequencing institutions.

Participants registered for the Ocean Sampling Day will need to take the following steps in order to comply with Access and Benefit-Sharing requirements set out in the Convention on Biological Diversity (see the section *ABS requirements* below) <u>AND</u> other requirements according to the International Law of the Sea (see the section *Law of the Sea permits* below).

Participants registered for the OSD will need to make sure that all required sampling permits are in place prior to the sampling event.

Step 0: Check in which maritime zone the sampling is planned: it can be in waters under national jurisdiction (case A: internal waters – territorial seas – exclusive economic zones); in areas beyond national jurisdiction (case B: the high seas and the deep seabed beyond the continental shelf); or in the Antarctic Treaty area (case C: the Antarctic).
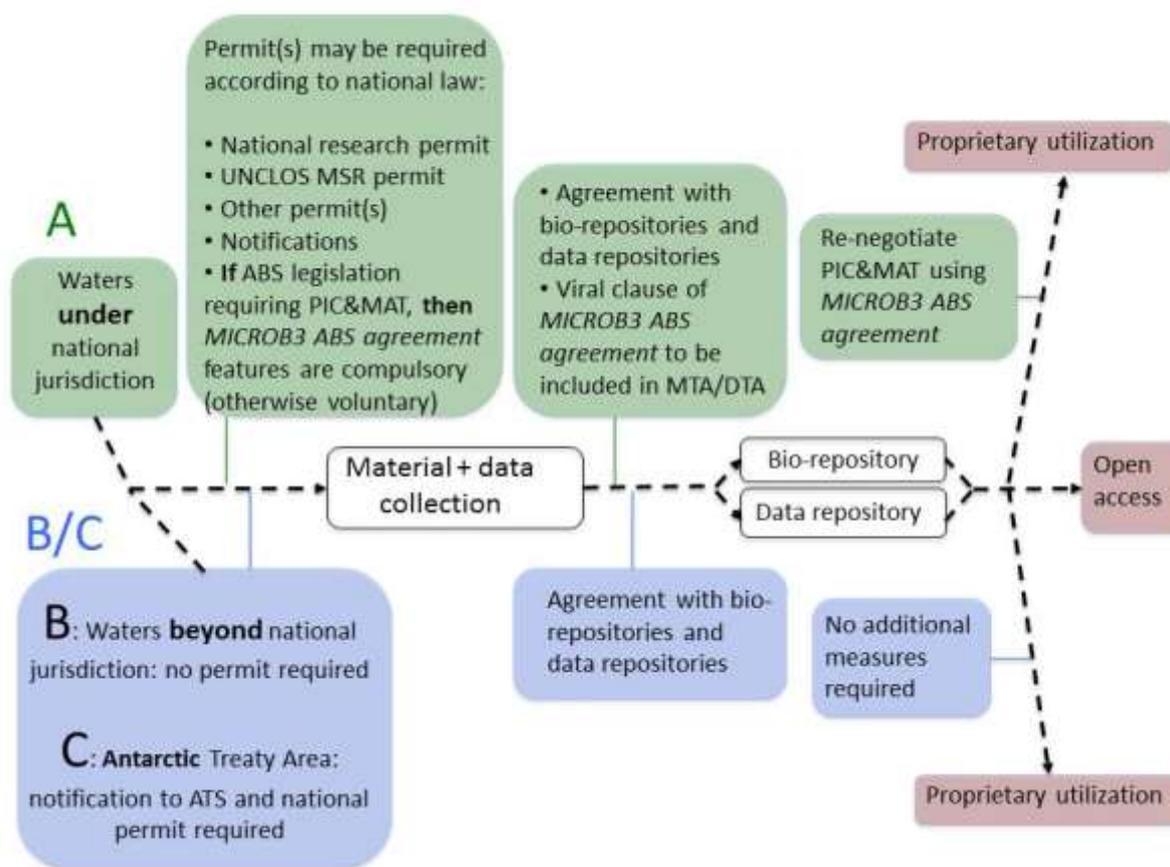
Figure 3. Overview of legal documents needed prior to the OSD as requested by national and international laws.
Sampling in waters under national jurisdiction (case A); sampling in waters beyond national jurisdiction (case B) and in the Antarctic Treaty Area (case C).

4.1.1. Case A: sampling in internal waters, territorial seas or exclusive economic zones

ABS requirements

If you are planning to sample in internal waters, territorial seas and/or exclusive economic zones, regardless of whether you are sampling in a foreign country or your own country (the country of your research institution), you need to take the following steps:

Step 1: As soon as possible, notify the Primary National Focal Point for the Convention on Biological Diversity (CBD NFP) of the country where you plan to sample (Provider State) and ask if any additional steps need to be taken. Contact details of the CBD NFP of the Provider State can be found at www.cbd.int/information/nfp.shtml.

Step 2: Ask for advice on the specific requirements to be fulfilled prior to sampling activities according to the Provider State's legislation on ABS.
Note: Such requirements may include a simple notification of the sampling and/or an ABS agreement.

Note: Basic information on national ABS legislations and measures can be found at www.cbd.int/abs/measures/.

Step 3: If the Provider State has ABS legislation requiring Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT), contact the Provider State's competent authority for ABS (as indicated by the CBD NFP) and start negotiating an ABS agreement on the basis of the Micro B3 ABS Model Agreement (see the Chapter 4.2).
Note: The Micro B3 ABS Model Agreement can be accepted and signed by the Competent National Authority on ABS as it is; or it can serve as the basis for negotiating a new agreement; or the Competent National Authority on ABS may propose its own model agreement.
Note: In the latter cases, the main features of the Micro B3 ABS Model Agreement (viral license clause, Art. 5.1; and renegotiation clause in case of change of intent, Art. 4.4) that are compulsory for complying with the OSD guidelines have to be integrated into the new/proposed ABS agreement. If these main features are not accepted, opt for not sampling in that particular country.
Note: If you need further advice please contact the **Micro B3 ABS help desk** at abs-helpdek@microb3.eu

Step 4: Access and use the material only in accordance with the requirements set out in the permit and/or the ABS agreement.

Step 5: Transfer the sampled material and/or the associated knowledge (which includes data and scientific results) to a third party only in accordance with the requirements set out in the permit/ABS agreement.
Note: **You need to be absolutely sure that you have the right to transfer the material and/or associated knowledge before sending the material and data to the OSD Core Team or to a third party**. If the permit/agreement is silent on this, it does not mean that you have the right to transfer. In the latter case, a clarification with the Provider State might be necessary.

Step 6: If you do not have the right to transfer or you want to use the material and/or associated knowledge for other purposes than the ones agreed upon in the ABS agreement/permit you obtained (change of intent), go back to the relevant authorities of the Provider State and renegotiate.

Step 7: Before transferring the collected material and/or associated knowledge to the OSD Core Team, sign a material transfer agreement (MTA) according to the Ocean Sampling Day practice.
Note: The MTA has to contain the viral clauses of the Micro B3 ABS Model Agreement (Article 5.1, 5.2).
Note: The bio-repository institution needs to agree with the depositing institution on the standards to be respected and on the legal status of the deposited materials.

Law of the Sea permits

According to the Law of the Sea you need a research permit/to obtain the consent of the coastal state in order to undertake Marine Scientific Research in the territorial seas and exclusive economic zones.
If you are planning to sample in:

a) In your own national waters (of the country of your research institution) contact the authority who is competent to release such a permit (it is usually the Ministry of Environment or the Ministry of Research or the Ministry of Transports) and provide full details of the research project

b) In a foreign country's waters contact the Embassy of the Provider State and provide full details of the research project. The Embassy should assist in obtaining the necessary consent from the competent authority

Note: It might be the case that different ministries are involved in releasing the permit.
Note: For marine protected areas there might be further restrictions.

4.1.2 Case B: sampling in the areas beyond national jurisdiction
No permits required.

4.1.3 Case C: sampling in the Antarctic Treaty Area
Any activity undertaken in the Antarctic Treaty area (south of 60º latitude) is subject to prior notification. Moreover the national legislation of the country undertaking the research might require applying and obtaining a permit.
Note: if you wish to enter and sample in a "Special Protected Area" you need a special permit: basic information can be found here http://www.ats.aq/e/ep_protected.htm

4.2. Micro B3 Model Agreement on Access to Marine Microorganisms and Benefit-Sharing

Micro B3 has developed a **Model Agreement on Access to Marine Microorganisms and Benefit Sharing (Micro B3 ABS Model Agreements)**, available in the **Annex IV** to be signed between the Provider State Institution and the Recipient Institution. The Micro B3 ABS Model Agreement (available for download along with the commentary at the link https://www.microb3.eu/news/commentary-micro-b3-abs-model-agreement)   is   in compliance with the Nagoya Protocol and the Convention on Biological Diversity that entered into force on 29 December 1993. The CBD has 3 main objectives:

1. The conservation of biological diversity
2. The sustainable use of the components of biological diversity
3. The fair and equitable sharing of the benefits arising out of the utilization of genetic resources

4.2.1. Possible purposes of the Micro B3 ABS Model Agreement

The Micro B3 ABS Model Agreement applies to public domain, hybrid and full commercial use at the point of access. This agreement can cover three situations:

(A) PUBLIC DOMAIN: The recipient envisions only public domain uses of genetic resources when you access the resource. Therefore, only conditions for public domain uses are negotiated at the moment of first access (article 4.2.). If needed commercial uses can still be envisioned in a later stage of the research process. Such commercial uses are permitted, but the conditions of this should be negotiated at the point of change in intent (consent clause under article 4.4).

(B) HYBRID: The recipient envisions public domain uses of some genetic resources/some use of genetic resources and already knows at the point of access some potential commercial uses for other genetic resources/other uses of the accessed genetic resource.

(C) PURE COMMERCIAL: The recipient envisions commercial uses for all the genetic resources accessed and decides to negotiate the benefit sharing conditions for commercial uses upon the access of the genetic resources. In this case only article 4.3. if the model contract applies (delete articles 4.2 – 4.4).

Note: Only the public domain and the hybrid Agreement are compatible with the OSD Data Sharing Policy.

4.2.2. Core benefits

By signing the Agreement, the provider country gives the research consortium the permit to sample in its seas and enters into a partnership. In such partnership some mutual benefits are automatically included and others can be decided/negotiated while signing the Agreement (in the specific conditions of article 4). These benefits are summarized below:

Automatic benefits from the contract (for the Provider country)
- Access to scientific results and data through open access integrated databases
- Additional monetary benefits in case of proprietary use as specified in the contract
- Benefits from the legal certainty provided by the agreement
- Be part of a major international scientific bio-informatics network

List of additional specific benefit-sharing items which can be agreed on upon accessing the sample in the specific provisions of article 10:
(a) related to the sampling

- Mentoring of provider country scientists by MICRO B3 project scientists that provide information and training on sampling and sampling processing

- Participation of the provider country scientists in all the scientific research activities on the boat and on land related to the sampling activity and its analysis
- Archiving of the sampling for a certain period of time
- Possible support for finding sequencing partners/preferred long-term archive partners

(b) related to the processing

- Possible collaboration between institutes

- Support in fund raising from national funding agencies for sequencing (as an option, for a limited number of samples, additional support from the MICRO B3 consortium

can be envisioned if there are major capacity gaps)

(c) related to data management, integration and access

- Possible participation in training for capacity building on bioinformatics, data management and data analysis (Micro B3 summary schools and workshops)

4.2.3. Core elements of the Micro B3 Agreement
Article 4.2 on non-commercial use (public domain uses, with renegotiation in case of change of intent)

Within the framework of upstream basic research, the Agreement allows using the collected resources for the public domain (article 4.2.). The application of this clause implies that the knowledge resulting from research and development on the collected materials has to be publicly available to the maximum possible extent. When patent rights are granted permission has to be obtained from the collection and the provider of the genetic material. There should be no expenses to accede to the knowledge acquired from the material accessed (apart from normal costs for dissemination), therefore every scientist with the adequate expertise will be able to access the scientific knowledge/information resulting from the project.

The agreement includes a renegotiation clause in case of change in intent from non-commercial use to commercial use. This clause is activated when the produced knowledge is used with exclusive protection, including products and processes developed. In those situations, the scientist/scientific institution will have to seek the consent of the provider country and to negotiate benefit-sharing.

Article 5: Viral license clause for improved monitoring, with consent procedure upon transfer to third parties

The viral licence concept means that the contract travels with the resource and the data upon transfer.

The Agreement offers a viral license clause in article 5. This clause guarantees that all the obligations of the initial ABS Agreement will be imposed on subsequent use of the materials and the produced data when transferred. When the viral licence clause is used, the scientist/scientific institution is allowed to transfer the material to third parties if the third parties sign a new contract, in which they commit themselves to respect the conditions of the initial ABS Agreement. Every transfer to new third parties will require the signature of a Material Transfer Agreement (MTA) that makes the initial ABS Agreement binding. The initial ABS Agreement should be attached to the MTA as an annex. At each transfer however, according to the Nagoya Protocol, consent is required from the competent national authority in the provider country (PIC or Prior Informed Consent). Here two situations can be distinguished:

- If you use the viral license clause, a notification to the competent national authority can be considered as the required prior informed consent.

- In case of modifications of contractual conditions, new consent has to be obtained from the competent national authority.

Article 1.3: Other implementation issues

For increasing legal certainty and facility of use, a copy of the contract will be made available to the registered users of the Micro B3 information system.

5. OSD Sampling Instructions & Protocols

5.1. Environmental Parameters

OSD participants are encouraged to measure as many environmental and biodiversity parameters as possible, based on their expertise and opportunities in order to ensure that ecologically meaningful knowledge can be derived from the OSD sampling effort.

Since OSD is a global sampling campaign with very divergent scientific interests of contributing OSD sampling Sites, Micro B3 has developed a **Micro B3 list of OSD mandatory and recommended ENVIRONMENTAL PARAMETERS**, **Table 1,** selected for a description of the OSD sample environment.

The Micro B3 list of OSD mandatory and recommended ENVIRONMENTAL PARAMETERS is a result of the Micro B3 consortium effort to find a consensus between hypothesis-driven and current best practice-driven approach to marine sampling.

The Micro B3 Use Case Workshop, organised at the EMBL-EBI, UK, in April 2012, identified several scientific use case studies from the area of diatom biology and from the area of marine prokaryotic biodiversity. From these studies we have extracted a hypothesis-driven candidate checklist of environmental parameters sampled for in the use case studies in order to answer scientific questions postulated in the studies. The candidate checklist has been reported in the Micro B3 deliverable 4.1, http://www.microb3.eu/sites/default/files/deliverables/MB3_D4_1_PU.pdf.

The Micro B3 Sampling Groups (SG) Workshop, organised at the EMBL-EBI, UK, in July 2012, addressed current and best practice in marine sampling. Participants from thirteen Institutes (BAS, CNRS, CIESM, CSIC, EMBL-EBI, EMPA, HCMR, JacobsUni, MARIS, UniHB , MBA, UOXF, VLIZ) discussed variation vs. consistency in capturing and processing data and as follow up ten groups participated in the Sampling Sites Survey, where detailed analysis of current sampling practices has been further investigated. Both the SG Workshop and the Survey were reported in the Micro B3 deliverable 4.2, http://www.microb3.eu/sites/default/files/deliverables/MB3_D4_2_PU.pdf.

We strongly encourages all OSD-participating research stations and cruises to measure as many of the ENVIRONMENTAL PARAMETERS (Table 1) as possible.

Table 1: Micro B3 list of OSD <u>mandatory</u> (highlighted) and <u>recommended</u> ENVIRONMENTAL PARAMETERS describing the marine environment of a Sample. Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

	CATEGORY	PARAMETER	DESCRIPTION	Control vocabulary/format *
ENVIRONMENTAL PARAMETERS	CTD	Conductivity	Electrical conductivity of water	SDN:P02:75:CNDC SDN:P06:46:UECA for mS/cm
		Temperature	Temperature of water	SDN:P02:75:TEMP SDN:P06:46:UPAA for °C
		Depth (m)	Vertical spatial coordinates	SDN:P02:75:AHGT SDN:P06:46:ULAA for m
		Salinity	Salinity of water	SDN:P02:75:PSAL SDN:P06:46:UGKG for PSU
		Fluorescence	Raw (volts) or converted (mg Chla/m^3) fluorescence of the water	SDN:P02:75:FVLT SDN:P06:46:UVLT for volts
	Seawater Nutrients Concentration	Nitrate	Nitrate concentration parameters in the water column	SDN:P02:75:NTRA SDN:P06:46:UPOX for µmol/L
		Nitrite	Nitrite concentration parameters in the water column	SDN:P02:75:NTRI SDN:P06:46:UPOX for µmol/L
		Phosphate	Phosphate concentration parameters in the water column	SDN:P02:75:PHOS SDN:P06:46:UPOX for µmol/L
		Silicate	Silicate concentration parameters in the water column	SDN:P02:75:SLCA SDN:P06:46:UPOX for µmol/L
		Ammonium	Ammonium concentration parameters in the water column	SDN:P02:75:AMON SDN:P06:46:UPOX for µmol/L
	Seawater Chemical Properties	pH	Alkalinity, acidity and pH of the water column	SDN:P02:75:ALKY
		Dissolved oxygen concentration	Dissolved oxygen parameters in the water column	SDN:P02:75:DOXY SDN:P06:46:KGUM for µmol/kg
	Seawater	Downward PAR	Visible waveband radiance and irradiance	SDN:P02:75:VSRW

CATEGORY	PARAMETER	DESCRIPTION	Control vocabulary/format *
Optical Properties		measurements in the water column	SDN:P06:46:UMES for µE/m^2/s
	Turbidity	Transmittance and attenuance of the water column	SDN:P02:75:ATTN SDN:P06:46:USTU for FTU or NTU
Organic Matter Concentration (Amount or Mass)	Carbon organic particulate (POC)	Particulate organic carbon concentration in the water column	SDN:P02:75:CORG SDN:P06:46:UGPL for µg/L
	Nitrogen organic particulate (PON)	Particulate organic nitrogen concentration in the water column	SDN:P02:75:NTOT SDN:P06:46:UGPL for µg/L
	Carbon organic dissolved (DOC)	Dissolved organic carbon concentration in the water column	SDN:P02:75:DOCC SDN:P06:46:UPOX for µmol/L
	Nitrogen organic dissolved (DON)	Dissolved organic nitrogen concentration in the water column	SDN:P02:75:TDNT SDN:P06:46:UMGL for mg/L
Organism Concentration (Amount, Volume or Mass)	Pigment concentrations	Concentration of pigments (e.g. chlorophyll a) extracted and analysed by fluorometry or HPLC	SDN:P02:75:CPWC SDN:P06:46:UMMC for mg/m^3
	Picoplankton (Flow Cytometry)	Abundance of cells in the water column (+other avail. cell properties)	SDN:P02:75:BATX SDN:P06:46:UPMM for #/m^3
	Nano/Microplankton	Abundance of cells in the water column (+other avail. cell properties)	SDN:P02:75:MATX or PATX SDN:P06:46:UPMM for #/m^3
	Meso/Macroplankton	Abundance of individuals in the water column (+other avail. properties)	SDN:P02:75:ZATX SDN:P06:46:UPMM for #/m^3
Community Production Rate	Primary Production (isotope uptake)	Primary Production in the water column	SDN:P02:75:PPRD SDN:P06:46:UGDC for mg/m^3/d
	Primary Production (oxygen)	Primary Production in the water column	SDN:P02:75:PPRD SDN:P06:46:UGDC for mg/m^3/d
	Bacterial production (isotope uptake)	Bacterial production in the water column	SDN:P02:75:UPTH SDN:P06:46:UGDC for mg/m^3/d

E N	CATEGORY	PARAMETER	DESCRIPTION	Control vocabulary/format *
		Bacterial production (respiration)	Bacterial production in the water column	SDN:P02:75:UPTH SDN:P06:46:UGDC for mg/m^3/d

* SDN:P02:75:xxxx is a controlled Terms list describing "WHAT" is measured. (http://www.seadatanet.org/urnurl/SDN:P02:75:XXXX)

* SDN:P06:46:xxxx is a controlled Terms list describing "UNITS" of measurements. (http://www.seadatanet.org/urnurl/SDN:P06:46:XXXX)

5.2. OSD App

The OSD App provides the opportunity to enter environmental parameters plus an illustrative photo in an electronic "logsheet" which are send to the OSD server. We strongly encourage all OSD participants to make use of the OSD App during the OSD sampling event. This will connect the community online and helps us to keep track of the sampling progress. All data submissions can be monitored in real time at https://mb3is.megx.net/osd-app/samples. Therefore we strongly recommend all samplers to use the App. It is available for Android and iPhone.

Steps to be taken:
1. Installation and fist time set-up
Download the OSD App for free in the Google Play Store or App Store. Search for "OSD citizen" keywords in the Google Play store (https://play.google.com/store/apps/details?id=com.iw.esa&hl=en) or Apple App store (https://itunes.apple.com/us/app/osd-citizen/id834353532?mt=8) .

After installation, please open the App while you still have internet connection as the App itself needs to download basic settings before you can create a new sample. This is only necessary once at the first start-up. Afterwards you don't need a network connection to use the App as it stores all the data offline until you next get internet access and click 'Upload Samples'.

Next, click on the "Personal" button on the left corner at the bottom - here it is mandatory to enter your first and last name. You can change this information as often as you like just do not forget to click the "Save" button at the bottom of the screen. Here you can also change the language.
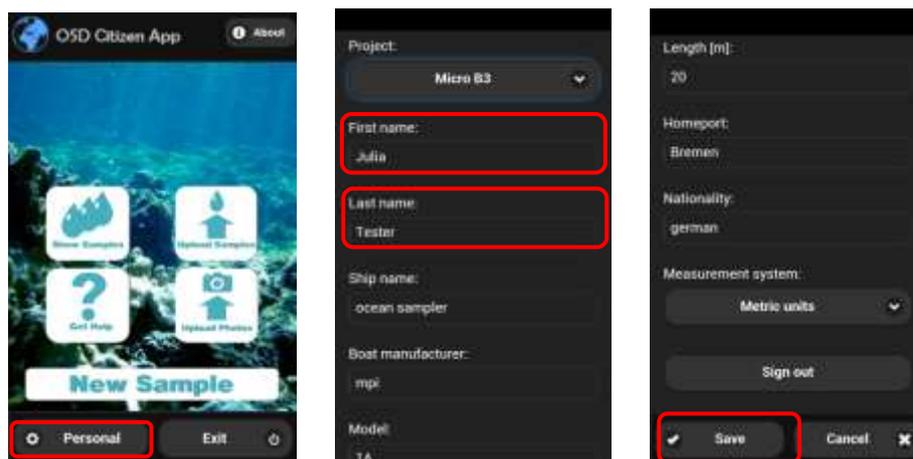
Please note: Your first and last name will be publicly displayed on the OSD Server website. If you do NOT want this, please choose a nickname.

For later data upload you have to log in with your Micro B3-IS user account or log in via your Google, Facebook or Twitter account. We recommend to generate your Micro B3-IS user account using your PC or Mac at https://mb3is.megx.net/register

Now you are ready to go!

2. Sampling
Create a new sample by clicking on the "New Sample" Button and fill in as much information as you have.
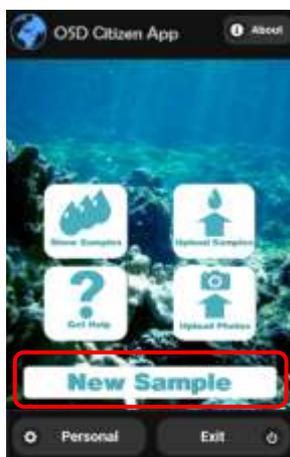
Mandatory fields are Time, Longitude, Latitude, GPS Accuracy (data will be automatically provided by your Smartphone if you have GPS enabled), Sampling depths and Sample name. Please enter your OSD site ID e.g. OSD102 in the field Sample name.
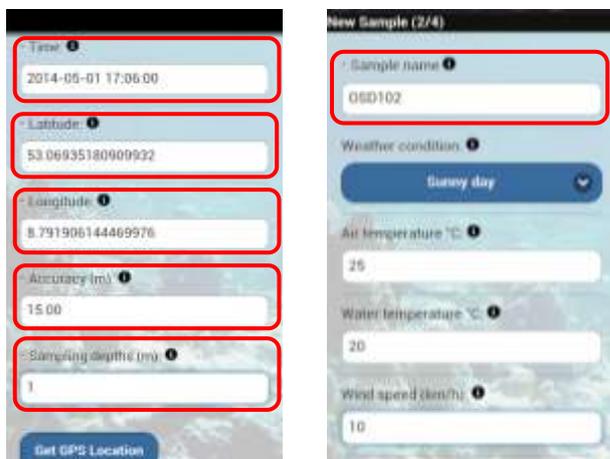
Please take a photo and upload it to the server. This will create a nice visual impression of the OSD campaign from all around the world.
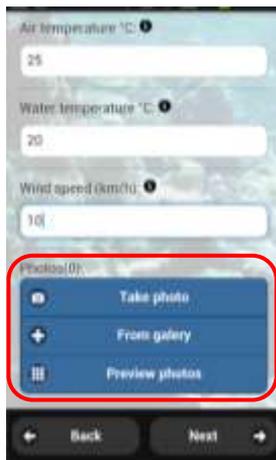
Continue to the last page and click "Done" to save the sample data on your phone. Upload the sample data and pictures to the OSD Server as soon as you have Internet connection.
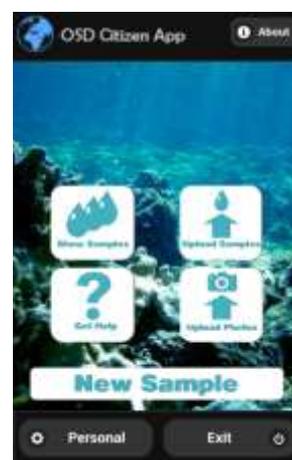
Finally, see your data and the data of your OSD Community on https://mb3is.megx.net/osd-app/samples:
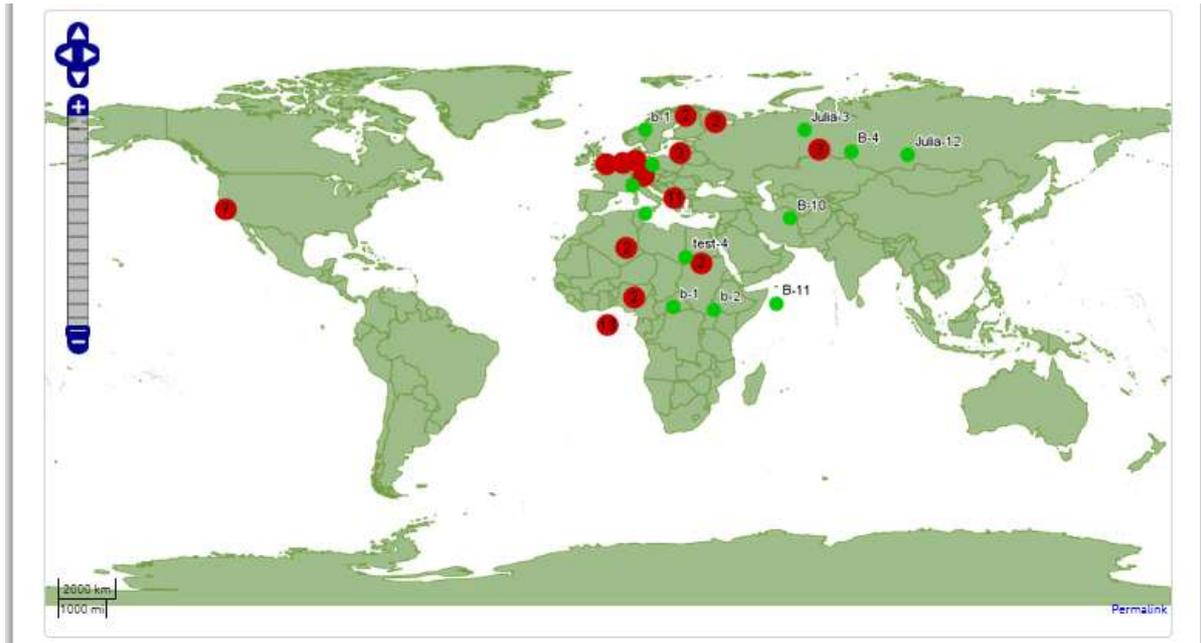
More information about our Citizen Science Project MyOSD as well as a video tutorial for the App can be found at http://www.microb3.eu/myosd

EyeOnWater-Colour App

Water colour is a result of substances that are either suspended or dissolved in the water column. Green colouration of the water is usually caused by microorganisms which carry out photosynthesis and are hence responsible for oxygen-production. In estuaries, the inflow of rivers to the sea with a lot of sediment and can be observed by brown colours. You can determine water colour in a rapid and easy way with the EyeOnWater-Colour App.
The App is freely available for Android Smartphones and iPhones.
https://play.google.com/store/apps/details?id=nl.maris.citclops.crosswalk
https://itunes.apple.com/us/app/eyeonwater-colour/id1021542366?mt=8.

Upload the EyeOnWater-Colour App
• You can immediately start to use the App.
• It is important to activate and receive a GPS signal during measurements.
• As soon as you have internet connection, data will be automatically uploaded to the server. You can conduct measurements without an internet connection.
• You can conduct measurements anonymously, or create a user account and log in for a more personalised experience. You can (but not need to) use your MyOSD nickname or MyOSD number.
• The App is available in English only.

User instructions are integrated into the App (How to use the App).
• You can measure water colour only if the bottom is not visible (otherwise colour will be determined by the substrate).
• In case the sun is shining, please try to have it in your back or side, to prevent sun-glint on your picture.
• You will get a good judgement on a good visibility of colour, soon, so do not worry.
• Please use the App on 21. June.
• In addition, you can use the App anytime before and/or after the 21 June.
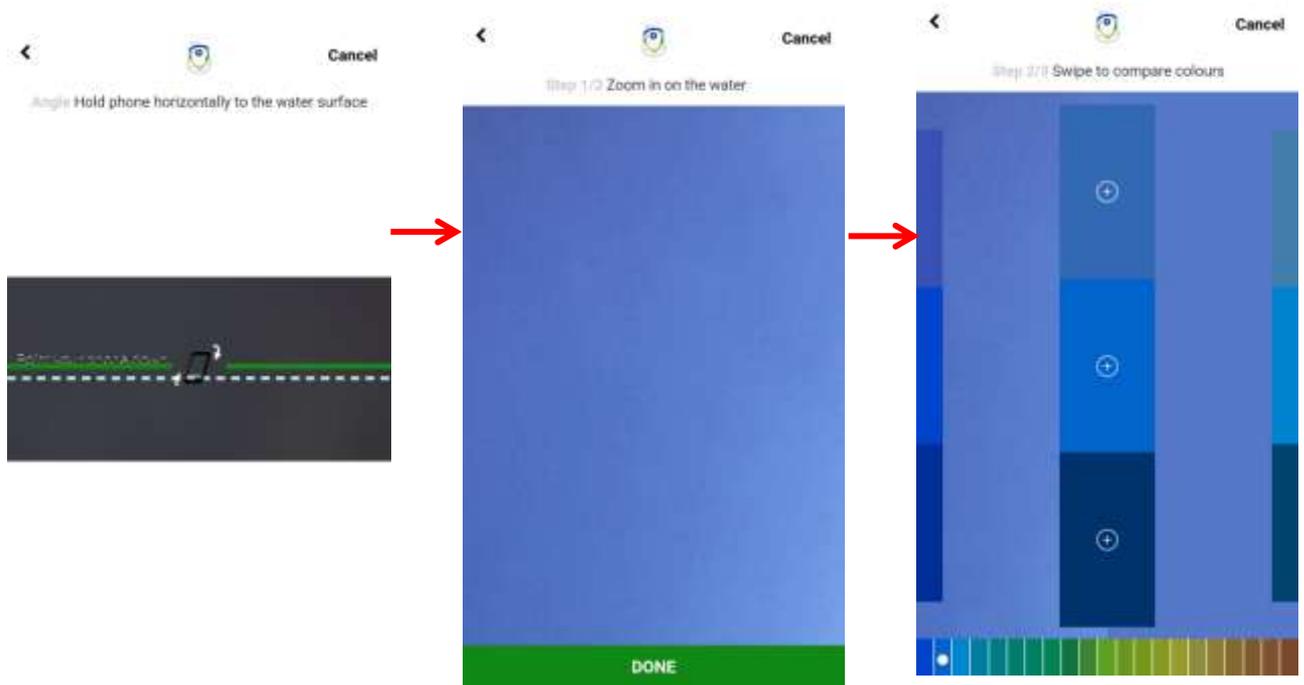
Keep your smartphone in a flat angle to take a photo of the water surface.

Take a photo of the water surface. Zoom into the part of the image, in which the water colour is represented best.

Choose the colour bar, which comes closest to the water colour on your image fraction. You can also directly compare the colour with the water. .

As soon as you connect your device to the internet, your measurement will be uploaded to the EyeOnWater server.
• You can view your measurement immediately on: www.EyeOnWater.org/color.
• Images from 21 June will be presented also on the MyOSD website.

Please answer three questions, press send and receive an automated feedback.

The EyeOnWater concept was developed within the EU FP7 project Citclops (citizens´ observatories for coast and ocean optical monitoring).

5.3. Samples for Genomics

This Handbook presents two protocols: (A) Collecting Prokaryotes on 0.22 µm filters using Sterivex cartridges; and (B) Collecting unicellular Eukaryotes on 0.8 µm membranes.

Sampling instructions and protocols were agreed upon by the Micro B3 consortium and correspond to standard sampling protocols used at the L4 station of the Western Channel Observatory (Protocol A) and during the Tara-Oceans expedition (Protocols B).

Protocol A is mandatory for participation in the main OSD event June 21st 2016. Micro B3/OSD participants are strongly encouraged to **take additional samples using protocol B**.

Samples obtained from protocols A will be bio-archived at the SI NMNH and **will be sequenced** (please see the Chapter 7).

IMPORTANT: **Please record any modification to the protocols on the OSD Logsheets described in the Annex I of this handbook.**

PROTOCOL (A)
OSD SAMPLING PROTOCOL FOR PROKARYOTES

Collecting Prokaryotes on 0.22 µm pore size filters using Sterivex cartridges

The Standard Operating Procedure (SOP) for collecting marine bacterial communities is based on the protocol used at the Western Channel Observatory by Gilbert et. al., (2010), PLoS ONE 5(11); http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0015545

1. Collect minimum of 4-6 bacterial subsamples (sterivex filter - replicates) for which at least the minimal reporting requirements are known. If possible, for each timezone take the samples between 10am and 2pm, ideally at local noon
   a. 4-5 Sterivex filter should be shipped to Germany for DNA extraction
   b. The remaining Sterivex filter should be stored at -80°C in your local freezer as backup

2. Isolate seawater using a Niskin bottle or 10% acid washed bucket from the surface (0-2m depth) of the water column. Please make sure that you have enough water for the 5-6 subsamples. We would recommend collecting 20-40 L of seawater.

3. Collect microbial community by passing the sampled seawater through a 0.22µm filter using Sterivex cartridges* available at (http://www.millipore.com/catalogue/item/svgv010rs). Please DO NOT perform a pre-filtration step. Please try to filter as much as possible until the filter clogs. *Instructions on how to use Sterivex filters can be viewed at the video of the MIRADA project at: http://amarallab.mbl.edu/mirada/mirada.html

4. Filtration using Sterivex filter should be done using either a peristaltic pump or a hand pump (e.g. 50mL sterile syringe). In either case you will need a leur lok adapter enabling an attachment to the Sterivex.
   a. You could also use another pump (e.g. vacuum pump), however, we would kindly ask you to write this deviation in the logsheet and online metadata form.

5. After the filtration the Sterivex should be pumped free of standing water following filtration but does not need to be dry. Please remove excess water by pumping air through the filter using the syringe

6. Seal the Sterivex filter using a sticky tac, e.g. blu tack or similar. Note that parafilm will crumble at -80°C and therefore should not be used.

7. Please label your filters as follows:

   <OSD_SiteID>_<Month>_<Year>_<SiteName>_<Protocol_Label>_<SampleNo>_<Depth>

i.e OSD3_06_16_Helgoland_NPL022_1_0m
i.e OSD3_06_16_Helgoland_NPL022_1_20m

    a. The protocol label for protocol A (sampling for prokaryotes) is NPL022
    b. Surface sampling correspond to a sampling depth of "0 m"
    c. It is important that your label contains both, OSD Site ID number and site name
        a. Examples on how to label your samples correctly are given below
    d. Please note that unclear labeling of samples results in rejection.

8. Protect you label from running. For example seal the label on the filter with transparent adhesive tape (also known as Scotch tape, Sellotape or Tesafilm). Another option would be to use temperature resistant labels (e.g. known as Tough-Tags).

9. Please store your labeled filter in a FREEZABLE plastic bag. Label the plastic bag according to (7). So in the end, your sample AND the plastic bag is correctly labeled. Please note that unclear labeling of samples results in rejection.

10. Freeze the plastic bag with the filters immediately in liquid nitrogen OR in a -80°C freezer. For short-term storage a -20°C freezer can be used. For transport from sea to the land for a period of time shorter than one hour samples can be stored in sealed bag buried in ice.

11. Please record the following details (per filter) on the logsheet:
    a. How much water you filtered
    b. Time taken to filter the sample
    c. Your observations about colour of the filter

12. Prepare your filters for shipping to Germany. All information can be found in the associated shipping protocol. Please note that the shipping deadline is 8th of July 2016. Therefore we would encourage all participants to prepare the shipping process in advance.

Relevant Metadata about the Sampling Protocol for Prokaryotes

List of "mandatory" and "optional" information for this sampling protocol, together with example values. These need to be written by hand for each sample in the SAMPLE section of the OSD Logsheets, and later typed in the SAMPLE section of the online OSD Metadata submission form (http://mb3is.megx.net/osd-registry/sample-registration)

(Mandatory)	SAMPLE_Title	OSD3_06_16_Helgoland_NPL022_1_0m
(Mandatory)	SAMPLE_Protocol_Label	NPL022
(Mandatory)	SAMPLE_Depth (m)	0 (surface)
(Optional)	SAMPLE_Quantity	2 L
(Optional)	SAMPLE_Fitration_Time	30 min
(Optional)	SAMPLE_Container	Sterivex
(Optional)	SAMPLE_Content	Particulate matter
(Optional)	SAMPLE_Size-Fraction_Upper-Threshold	no pre-filtration
(Optional)	SAMPLE_Size-Fraction_Lower-Threshold	0.22 μm
(Optional)	SAMPLE_Treatment_Chemicals	none
(Optional)	SAMPLE_Treatment_Storage	Liquid Nitrogen or -80°C

Examples from OSD 2014 - **CORRECT** labeling of samples:

UNACCEPTABLE examples from previous OSD pilot events

PROTOCOL (B)
Collecting unicellular Eukaryotes in the size-fraction >0.8 μm

ATTENTION: OSD 2016 will focus on Protocol A (Sterivex filtration). Due to limited resources we can NOT ACCEPT samples from Protocol B (LifeWatch cooperation focusing on Eukaryotic fraction). However, in case someone is interested to do some follow up studies in house, we kept the protocol B in the handbook for the sake of completeness.

The Standard Operating Procedure (SOP) for collecting unicellular Eukaryotes is based on protocols used during the Tara Oceans Expedition

1. Collect 20-100L of seawater using Niskin bottles or 10% acid washed bucket from surface water (0-2m depth). If possible, for each time zone, take the samples between 10am and 2pm, ideally at local noon. Filtration should start as soon as possible and no later than 2 hours after sampling, making sure to keep seawater in near-ambient conditions. Please make sure to gently homogenise the sample before filtration.

2. Additional depths such as a deep chlorophyll maximum, an oxygen minimum zone, or a benthic boundary layer may also be sampled for PROTOCOL B. However, priority will be given to the analysis of surface samples.

3. Measure all OSD <u>mandatory</u> information about SAMPLING, EVENTS, SAMPLES and ENVIRONMENT (see Table 2a in the OSD Handbook) and write them by hand on OSD Logsheets. Please use <u>separate</u> <u>logsheets</u> for PROTOCOLS A and B. Each logsheet can capture information about 6 replicates. Later, you will be asked to also fill <u>the online OSD Metadata submission form</u> (http://mb3is.megx.net/osd-registry/sample-registration) based on the information recorded by hand on the OSD Logsheets.

4. Rule regarding the required <u>pre-filtration</u>: DO NOT pre-filter seawater. After filtration, occasional large organisms (e.g. copepods) should be carefully picked from the filter membrane and discarded. When there are too many large organisms (e.g. >5), filtration should be done again with a 200 μm pre-filtration. In any case, whenever large organisms are picked from the membrane filters, please make a comment on the OSD Logsheet.

5. Rule regarding the required <u>filtration method</u>: Filtration should be done preferentially using a peristaltic pump. The flow rate will depend on the diameter of the filter. As a rule of thumb, adjust the rate to obtain a regular outflow occupying the full inner diameter of the tubing, i.e. not only dripping. The preferred filter type is a <u>polycarbonate membrane with a pore size of 0.8 μm</u>. The diameter of the membrane (e.g. 47 mm, 90 mm or 142 mm) is up to each OSD Site and should be selected based on the amount of material in the water and the intended filtration volume per filter, i.e. based on previous experience. When the preferred filter type (polycarbonate membrane with a pore size of 0.8 μm) is not available, we recommend using polycarbonate membranes or Sterivex with a pore size within the range 0.2-1.2 μm.

6. Rule regarding the required <u>filtration volume per replicate</u>: The filtration volume per replicate will vary depending on the diameter of the membrane filter (e.g. 47 mm, 90 mm or 142 mm) and on the amount of material in the water, typically ranging from 5 L to 50 L. As an indication that enough material was collected, filter membranes should be coloured.

7. Rule regarding the required <u>number of replicates</u>: Filter a minimum of two (2) and up to six (6) replicates per sampling depth. The number of replicates will vary depending on the diameter of the membrane filter (e.g. 47 mm, 90 mm or 142 mm) and on the amount of material in the water. Membranes should be replaced as soon as filtration appears to clog, thus adding replicates as you proceed with the filtration. You may filter for example 4x5 L, 2x10 L, 5x10 L, 5x20 L or 2x50 L. Replicates sent to Germany may be pooled for nucleotides extraction and one replicate may be sent for bio-archiving. Priority is given to extracting enough material for sequencing, so having more than 2 replicates increases the chances of bio-arching your sample.

8. Rule regarding the required <u>filtration time per replicate</u>: The <u>maximum filtration time per replicate</u> should be 60 minutes. Membranes should be replaced as soon as filtration appears to clog. As an indication that enough material was collected, filter membranes should be coloured. The SAMPLE_Filtration_time_(min) must be recorded for each replicate on the OSD Logsheet.

9. Rule regarding the required <u>storage</u>: Place each filter in a separate sample container (e.g. 5-50 mL <u>cryotubes</u>) and store them immediately in liquid nitrogen or in a -80°C freezer. A -20°C freezer can be used for temporary storage while filtering several replicates.

10. Please label your sample containers as follows (this label = SAMPLE_Title on the logsheet):
    <OSD_SiteID>_<Month>_<Year>_<SiteName>_<Protocol_Label>_<SampleNo>_<Depth>
    e.g. OSD3_06_16_Helgoland_NE08_1_surface
    e.g. OSD3_06_16_Helgoland_NE08_2_surface

11. Keep the filters in your laboratory for future use or in house studies. Due to limited resources we can NOT ACCEPT samples from Protocol B for OSD2016.
    Please store them in liquid nitrogen or in a -80°C freezer.

Relevant Metadata about the Sampling Protocol for Eukaryotes

List of "mandatory" and "optional" information for this sampling protocol, together with example values. These need to be <u>written by hand for each sample in the SAMPLE section of the OSD Logsheets</u>, and later <u>typed in the SAMPLE section of the online OSD Metadata submission form</u> (http://mb3is.megx.net/osd-registry/sample-registration).

(Mandatory)	SAMPLE_Title	OSD3_06_15_Helgoland_NE08_1_surface
(Mandatory)	SAMPLE_Protocol_Label	NE08
(Mandatory)	SAMPLE_Depth (m)	2 (surface)
(Optional)	SAMPLE_Quantity	20 L
(Optional)	SAMPLE_Fitration_Time	60 min
(Optional)	SAMPLE_Container	5 mL cryotube
(Optional)	SAMPLE_Content	Particulate matter on a 47 mm 0.8 μm pore size polycarbonate membrane filter
(Optional)	SAMPLE_Size-Fraction_Upper-Threshold	no pre-filtration
(Optional)	SAMPLE_Size-Fraction_Lower-Threshold	0.8 μm
(Optional)	SAMPLE_Treatment_Chemicals	none
(Optional)	SAMPLE_Treatment_Storage	Liquid Nitrogen

Polycarbonate Membrane Filter for Sampling:

Code no ATTP04700,   ISOPORE MEMBRANE,POLYCARBONATE, HYD POLYCARBONATE, HYDROPHILIC, 0.8 UM, 47 MM, WHITE, PLAIN - PKG 100 - FILTER MEMBRANES – POLYCARBONATE MEMBRANE (170 € for 100 filters)
http://www.millipore.com/catalogue/item/attp04700

Code no ATTP14250   ISOPORE MEMBRANE,POLYCARBONATE, HYD POLYCARBONATE, HYDROPHILIC, 0.8 UM, 142 MM, WHITE, PLAIN - PKG 50 - FILTER MEMBRANES – POLYCARBONATE MEMBRANE (320 € for 50 filers) –
http://www.millipore.com/catalogue/item/attp14250

6. OSD Mandatory and Recommended Information

6.1 OSD Mandatory Information

One OSD dataset is a collection of information elements from one OSD-participating cruise/station that describes the station/cruise, sampling events of the station/cruise, collected samples, recorded observational and derived parameters, biodiversity information, instruments and procedures for material and data collection and analysis.

A OSD dataset will consist of environmental data and biodiversity data derived from molecular and morphological analysis, and will be archived in several domain-specific archives. Each OSD dataset has to therefore contain a minimum core of classifiers allowing relating one otherwise disparate data type to another. This minimum contextual information of a sample will further be referred to as the MANDATORY OSD CHECKLIST.

The **MANDATORY OSD CHECKLIST**, **Table 2a**, is the minimum reporting standard for every Micro B3/OSD-collected sample ensuring that each OSD sample is accompanied by essential contextual data. The checklist can be reported by any OSD sampling Site irrespective of their scientific expertise or sampling opportunities.

The MANDATORY OSD CHECKLIST must be submitted to the OSD Information System submission portal at http://mb3is.megx.net/osd-registry/sample-registration.

The MANDATORY OSD CHECKLIST is included in the OSD Logsheets (please see the **Annex I**), a copy of which must be sent together with the OSD samples to the members of the OSD Core Team in the MPIMM in Bremen, Germany.

Table 2a: OSD checklist of OSD <u>mandatory</u> information about SAMPLING, EVENTS, SAMPLES and ENVIRONMENT of a Sample.
Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

	Field	Description	Control vocabulary/format*	Example
MANDATORY MICRO B3 CHECKLIST	SAMPLING_ Campaign	Refers to a finite or indefinite activity aiming at collecting data/samples, e.g. a cruise, a time series, a mesocosm experiment.	Free text	Micro B3-OSD2014
	SAMPLING_ Site	Refers to the unique identifier and name of the site/station where the data/sample collection is performed.	Format: <Site ID from OSD Site Registry >, <Site name from OSD Site Registry>	OSD5, Poseidon-E1-M3 Time Series Station
	SAMPLING_ Platform	Refers to the specific unique stage from which the sampling device was deployed; includes the platform category and platform name.	Format: <Platform category from SDN:L06>,<Platform name>	research vessel, FILIA
	EVENT_ Date/Time	Date and time when the sampling event started and ended, e.g. each CTD cast, net tow, or bucket collection is a distinct event.	Date and time in UTC; Format: yyyy-mm-ddThh:mm:ssZ	2013-06-21T14:05:00Z/ 2013-06-21T14:46:00Z
	EVENT_ Longitude	Longitude of the location where the sampling event started and ended, e.g. each CTD cast, net tow, or bucket collection is a distinct event.	Format: ###.###### Decimal degrees; East= +, West= - Format: Use WGS 84 for GPS data	035.666666 035.670200
	EVENT_ Latitude	Latitude of the location where the sampling event started and ended, e.g. each CTD cast, net tow, or bucket collection is a distinct event.	Format: ##.###### Decimal degrees; North= +, South= - Format: Use WGS 84 for GPS data	-24.666666 -24.664300
	SAMPLE_ Title	A short informative description of the sample. Must be unique for each sample, (i.e. for each filter generated during sampling).	Format: <OSD_SiteID>_<Month>_<Year>_<SiteName>_<Protocol_Label>_<SampleNo>_<Depth>	OSD3_06_14_Helgoland_NPL022_1_surface

	SAMPLE_Depth	The distance below the surface of the water at which a measurement was made or a sample was collected.	Format: ##.# Positive below the sea surface. SDN:P06:46:ULAA for m	1.5
	SAMPLE_Protocol_Label	Identifies the protocol used to produce the sample, e.g. filtration and preservation.	Term list; See the *SAMPLE_Protocol_Label* in the OSD Protocols Section for details	NPL022
	ENVIRONMENT_Biome	Descriptor of the broad ecological context of a sample.	Terms list: EnvO	ENVO:01000023 for "marine pelagic biome"
	ENVIRONMENT_Feature	Compared to biome, feature is a descriptor of a geographic aspect or a physical entity that strongly influences the more local environment of a sample.	Terms list: EnvO	ENVO:01000080 for "pelagic isothermal surface"
	ENVIRONMENT_Material	Descriptor of the material that was displaced by the sample, or material in which the sample was embedded, prior to the sampling event.	Terms list: EnvO	ENVO:00002225 for "mesotrophic water"
	ENVIRONMENT_Temperature	Temperature of water at the time of taking the sample.	Format: ##.# SDN:P02:75:TEMP SDN:P06:46:UPAA for °C	16.2 °C
	ENVIRONMENT_Salinity	Salinity of water at the time of taking the sample.	Format: ##.# SDN:P02:75:PSAL SDN:P06:46:UGKG for PSU	39.1 psu

*SDN:L06::XX is a controlled Terms list describing "CATEGORIES" of platforms.
(http://seadatanet.maris2.nl/v_bodc_vocab_v2/search.asp?lib=L06 for human interface)

* SDN:P02::XXXX is a controlled Terms list describing "WHAT" is measured.
(http://seadatanet.maris2.nl/v_bodc_vocab_v2/search.asp?lib=P02 for human interface)

* SDN:P06::XXXX is a controlled Terms list describing "UNITS" of measurements.
(http://seadatanet.maris2.nl/v_bodc_vocab_v2/search.asp?lib=P06 for human interface

* OSD Sites Registry is a controlled register for OSD sampling Sites (http://mb3is.megx.net/osd-registry)

* EnvO is Environment Ontology (http://www.environmentontology.org/Browse-EnvO)

6.2 OSD Recommended Information

The *mandatory OSD checklist* is the minimum contextual information associated with each OSD sample. It is a multi-disciplinary standard on a crossroad of three scientific domains, biodiversity, genomic and oceanographic, representing research on microbial biodiversity and function in the marine environment, **Figure 4**.
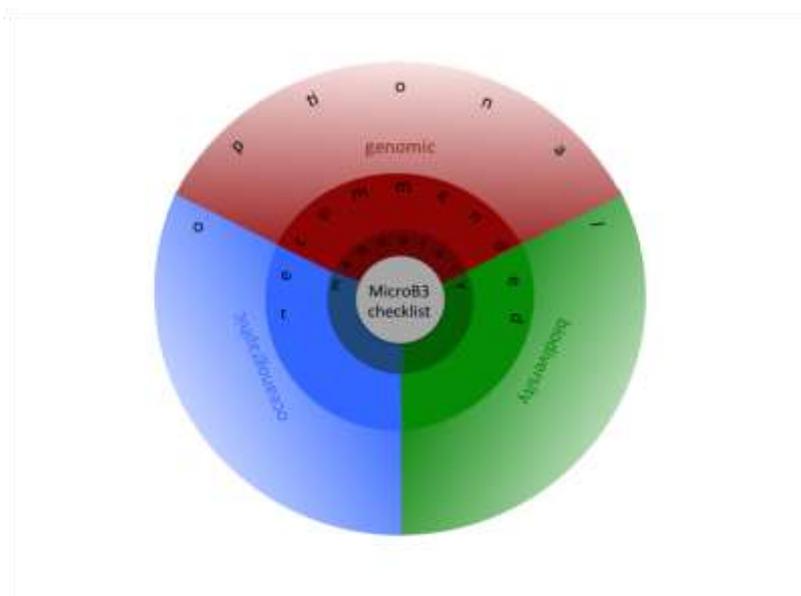
Figure 4: M*andatory OSD checklist* is in the centre of mandatory, recommended and optional information elements in standards of three scientific domains.

Although the *mandatory OSD checklist*captures essential contextual data of each OSD sample, OSD ConsortiumConsortium advises to report also the **MICRO B3-RECOMMENDED INFORMATION, Tables 2b – 2g**, which will offer a number of assets to the data providers:

a. Assures compliance of OSD datasets with the standards of all three domains (oceanographic, genomic and biodiversity), which means that OSD datasets will be consistent and meaningful to each of the scientific communities involved.
b. Allows OSD samples to be sequenced and molecular data deposited in a sequence data archive, the ENA.
c. Allows the PANAGEA to create a CDI-compliant discovery record from each OSD dataset, which means that the OSD dataset will be visible via the SeaDataNet to the global oceanographic data network and the Micro B3-developed bioinformatics infrastructure will be able to link the OSD dataset with additional relevant oceanographic ancillary observations, predictive models and climatology, thus enriching the environmental context of OSD samples significantly.
d. Allows the PANGAEA to create an OBIS-scheme-compliant record from each OSD dataset, which means that potential novel marine organisms identified from the OSD dataset will be available to the biodiversity community.

The OSD consortium strongly encourages OSD marine research stations and cruises to report recommended information on SAMPLING (**Table 2b**), EVENT (**Table 2c**), SAMPLE (**Table 2d**), ENVIRONMENT (**Table 2e**), ORGANISMS of a sample (**Table 2f**) and MEASUREMENT (**Table 2g**).

Table 2b: OSD checklist of OSD mandatory (highlighted) and recommended information about SAMPLING.
Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

<table>
<tr><th>Field</th><th>Description</th><th>Control vocabulary/format*</th><th>Example</th></tr>
<tr><td>SAMPLING_ Campaign</td><td>Refers to a finite or indefinite activity aiming at collecting data/samples, e.g. a cruise, a time series, a mesocosm experiment.</td><td>Free text</td><td>Micro B3-OSD2014</td></tr>
<tr><td>SAMPLING_ Site</td><td>Refers to the unique identifier and name of the site/station where the data/sample collection is performed.</td><td>Format: <Site ID from OSD Site Registry >, <Site name from OSD Site Registry></td><td>OSD5, Poseidon-E1-M3A Time Series Station</td></tr>
<tr><td>SAMPLING_ Platform</td><td>Refers to the specific unique stage from which the sampling device was deployed; includes the platform category and platform name.</td><td>Format: <Platform category from SDN:L06>,<Platform name></td><td>research vessel, FILIA</td></tr>
<tr><td>SAMPLING_ Investigators</td><td>List of people who will appear in the citation of data publications. Please order the list according to authorship. The first author is the contact person.</td><td>Format: <LASTNAME>, <FirstName>, <Institution>, <email></td><td>JONES, Peter, Institute1, pjones@institute1.eu; SMITH, Mary, Institute2, msmith@institute2.eu</td></tr>
<tr><td>SAMPLING_ Project</td><td>Refers to the project that organised/funded the data/sample collection.</td><td>Free text</td><td>Micro B3</td></tr>
</table>

INFORMATION ABOUT SAMPLING

	Field	Description	Control vocabulary/format*	Example
	SAMPLING_ Objective	Describes the scientific context/interest of the sampling activity. This information is useful to generate a short abstract as part of the data set citation.	Free text; 100-500 words	A short abstract

Table 2c: OSD checklist of OSD <u>mandatory</u> (highlighted) <u>and recommended</u> information about a sampling EVENT.
Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

	Field	Description	Control vocabulary/format*	Example
INFORMATION ABOUT A SAMPLING EVENT	EVENT_ Date/Time	Date and time when the sampling event started and ended, e.g. each CTD cast, net tow, or bucket collection is a distinct event.	Date and time in UTC; Format: yyyy-mm-ddThh:mm:ssZ	2013-06-21T14:05:00Z/ 2013-06-21T14:46:00Z
	EVENT_ Longitude	Longitude of the location where the sampling event started and ended, e.g. each CTD cast, net tow, or bucket collection is a distinct event.	Format: ###.###### Decimal degrees; East= +, West= - Format: Use WGS 84 for GPS data	035.666666 035.670200
	EVENT_ Latitude	Latitude of the location where the sampling event started and ended, e.g. each CTD cast, net tow, or bucket collection is a distinct event.	Format: ##.###### Decimal degrees; North= +, South= - Format: Use WGS 84 for GPS data	-24.666666 -24.664300
	EVENT_ Device	Refers to the instrument/gear used to collect the sample or the sensor used to measure environmental parameters.	Free text	10L-Niskins or 5L-Bucket

	Field	Description	Control vocabulary/format*	Example
	EVENT_ Method	Refers to the deployment procedure of the Device.	Free text	12 Niskins were deployed on a Rosette
	EVENT_ Comment	Report any deviation.	Free text	Lots of Jellyfish in the water

Table 2d: OSD checklist of OSD mandatory (highlighted) and recommended information about a SAMPLE.

Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

	Field	Description	Control vocabulary/format*	Example
	SAMPLE_ Title	A short informative description of the sample. Must be unique for each sample, (i.e. for each filter generated during sampling).	Format: <OSD_SiteID>_<Month>_<Year>_<SiteName e>_<Protocol_Label>_<SampleNo>_<Depth >	OSD3_06_14_Helgola nd_NPL022_1_surface
INFORMATION ABOUT A SAMPLE	SAMPLE_ Depth	The distance below the surface of the water at which a measurement was made or a sample was collected.	Format: ##.#; Positive below the sea surface. SDN:P06:46:ULAA for m	1.5
	SAMPLE_ Protocol_Label	Identifies the protocol used to produce the sample, e.g. filtration and preservation.	Term list; See the *SAMPLE_Protocol_Label* in the OSD Protocols Section for details	NPL022
	SAMPLE_ Quantity	Refers to the quantity of environment that was sampled, most often with dimensions Length, Amount, Mass or Time.	Format : ###.### in litres See the *SAMPLE_Quantity* in the OSD Protocols Section for details	20

SAMPLE_ Container	Refers to the container in which the sample is stored prior to analysis.	Term list; See the *SAMPLE_Container* in the OSD Protocols Section for details	Sterivex cartridge
SAMPLE_ Content	Refers to the content of the sample container. While the sample might target bacteria, the sample content might be a filter or a volume of water.	Term list; See the *SAMPLE_Content* in the OSD Protocols Section for details	Particulate matter on a 0.22 µm pore size filter
SAMPLE_ Size-Fraction_ Upper-Threshold	Refers to the mesh/pore size used to pre-filter/pre-sort the sample. Materials larger than the size threshold are excluded from the sample.	Term list; See the *SAMPLE_Size-Fraction_Upper-Threshold* in the OSD Protocols Section for details; in µm	no pre-filtration
SAMPLE_ Size-Fraction_ Lower-Threshold	Refers to the mesh/pore size used to retain the sample. Materials smaller than the size threshold are exclude from the sample.	Term list; See the *SAMPLE_Size-Fraction_Lower-Threshold* in the OSD Protocols Section for details; in µm	0.22
SAMPLE_ Treatment_ Chemicals	Refers to the chemicals added to the sample, in the container, preservatives.	Terms list: ChEBI; See the *SAMPLE_Treatment_Chemicals* in the OSD Protocols Section for details	None
SAMPLE_ Treatment_ Storage	Refers to the conditions in which the sample is stored, e.g. temperature, light conditions, time.	Term list; See the *SAMPLE_Treatment_Storage* in the OSD Protocols Section for details	-80 degrees Celsius

* ChEBI is an ontological classification and dictionary of small chemical compounds (http://www.ebi.ac.uk/chebi/init.do)

Table 2e: OSD checklist of OSD <u>mandatory</u> (highlighted) <u>and recommended</u> information about ENVIRONMENT of a Sample.
Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

Field	Description	Control vocabulary/format*	Example
ENVIRONMENT_ Biome	Descriptor of the broad ecological context of a sample.	Terms list: EnvO	ENVO:01000023 for "marine pelagic biome"
ENVIRONMENT_ Feature	Compared to biome, feature is a descriptor of a geographic aspect or a physical entity that strongly influences the more local environment of a sample.	Terms list: EnvO	ENVO:01000080 for "pelagic isothermal surface"
ENVIRONMENT_ Material	Descriptor of the material that was displaced by the sample, or material in which a sample was embedded, prior to the sampling event.	Terms list: EnvO	ENVO:00002225 for "mesotrophic water"
ENVIRONMENT_ Temperature	Temperature of water at the time of taking the sample. Define the parameter according to Table 2g.	Format: ##.# SDN:P02:75:TEMP SDN:P06:46:UPAA for °C	16.2 °C
ENVIRONMENT_ Salinity	Salinity of water at the time of taking the sample. Define the parameter according to Table 2g.	Format: ##.# SDN:P02:75:PSAL SDN:P06:46:UGKG for PSU	39.1 psu
ENVIRONMENT_ Marine_Region	It characterises the environment, based on the latitude and longitude, by reference to geographic, political, economic or ecological boundaries.	Terms list: Marine Regions	Crete Sea
ENVIRONMENT_ Other_Parameters	Add as many fields as there are other environments parameters measured. Define the parameter according to Table 2g. See list of recommended environmental parameters in Table 1		

* Marine Regions is a standard list of marine georeferenced place names (http://www.marineregions.org/)

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Table 2f: OSD checklist of OSD mandatory (highlighted) and recommended information about ORGANISMS of a Sample.
Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

	Field	Description	Control vocabulary/format*	Example
INFORMATION ABOUT THE ORGANISMS OF A SAMPLE	ORGANISM_ Taxon_ID	An identifier for the nomenclatural (not taxonomic) details of a scientific name.	Terms list: WoRMS Format: LSID	urn:lsid:marinespecies.org:taxname: 345516
	ORGANISM_ Taxon_Scientific_Name	The full name of the lowest level taxon.	Terms list: WoRMS Format: Taxon name	Prochlorococcus marinus
	ORGANISM_ Sex	The sex of a specimen or collected/observed individual(s).	Terms list: M=Male; F=Female; H=Hermaphrodite; I=Indeterminate (examined but could not be determined; U=Unkown (not examined); T=Transitional (between sexes; useful for sequential hermaphrodites); B = Both Male and Female	M
	ORGANISM_ Life-Stage	Indicates the life stage present.	Free text	ND
	ORGANISM_ Measurement_ Size	Refers to size measurements that are made concurrently to the enumeration and identification of organisms. Define the parameter according to Table 2g.		
	ORGANISM_ Measurement_ Biovolume	Refers to volume measurements/calculations that are made concurrently to the enumeration and identification of organisms. Define the parameter according to Table 2g.		
	ORGANISM_ Measurement_ Biomass	Refers to biomass measurements/calculations that are made concurrently to the enumeration and identification of organisms. Define the parameter according to Table 2g.		

* WoRMS is a World Register of Marine Species (http://www.marinespecies.org/aphia.php?p=search)

Table 2g: OSD checklist of OSD <u>mandatory</u> (highlighted) <u>and recommended</u> information about a MEASUREMENT
Recommendation applies to OSD marine research cruises and stations sampling in the pelagic zone.

	Field	Description	Control vocabulary/format*	Example
INFORMATION ABOUT A MEASUREMENT	PARAMETER_ID	Unique ID from a controlled vocabulary.	SDN:P02:75:xxxx	SDN:P02:75:CORG for Particulate organic carbon concentration in the water column
	PARAMETER_ Name	Common name for the parameter.	Free text	Biomass
	QUANTITY	Describes the quantity measured using terms from the Système International of units.	Free text; SI of units	Mass concentration
	DIMENSIONS	Describes the quantity measured using dimension terms from the Système International of units.	Free text; SI of units	M^1 L^-3
	CURRENCY	May often refer to a TAXONOMY_ID or a CHEMICAL_ID.	Free text; Terms list: WoRMS; Terms list: ChEBI	Organic carbon
	UNITS	Describes the units of the quantity measured using terms from the Système International of units.	SDN:P06:46:xxxx	SDN:P06:46:UMGL for mg/L
	METHOD	Describes the method used. Equivalent to methodological details provided in a paper.	Free text	Mass spectrometry
	COMMENT	Any comment about the measurement.	Free text	Inorganic carbon removed by acidification

7. Handling OSD samples (shipping, sequencing and bio-archiving)

7.1. Shipping and sequencing OSD samples

OSD samples will be available for sequence analysis. OSD sampling stations and cruises shall **send 4-5 OSD samples from the protocol A** to members of the OSD Core Team at the Max Planck Institute for Marine Microbiology, Germany.

OSD sampling Sites are advised to keep any additional replicates locally following storage conditions as described in the OSD sampling protocol A.

IMPORTANT: Please note that **each OSD sample must be accompanied by** contextual information completed in **the OSD Logsheets** (please see **Annex I**).

The OSD Consortium reserves the right not to release sequences of those OSD samples lacking metadata of the *mandatory OSD checklist*.

Shipping deadline for June 2016 OSD samples is 8th **of July 2016.**
Please note that the OSD Core Team cannot guarantee DNA extraction and subsequent nucleotide sequence analysis for samples arriving after the shipping deadline.

The OSD Core Team will upon receipt of the OSD samples and all above listed documents perform the following tasks (depending on funding availability):

1. Assign each sample (i.e. each individual filter) a unique Sample ID.
2. Extract DNA from OSD samples following standardised protocol.
3. Send extracted DNA of prokaryotic OSD samples to the LGC Genomics GmbH, Germany, for nucleotide sequence analysis of prokaryotes.
4. Send a replicate of each OSD sample with mandatory metadata to the SI NMNH for bio-archiving.

The LGC Genomics GmbH will perform (depending on funding availability):

1. 16S rRNA gene sequencing. Microbial community profile of up to 500 OSD samples will be analysed in one Illumina MiSeq V3 run (2 x 300bp) = 2 x 40,000 Reads per sample.
2. Sequencing of metagenomic DNA from 150 OSD samples in eight Illumina MiSeq V3 runs (2 x 300bp) = 2 x 1,000,000 Reads per sample. Priority here will be given to the OSD samples that are highly contextualized with measured environmental parameters.

Generated sequence data will be sent back to the MPIMM in Bremen, Germany.

OSD 2016 SHIPPING PROTOCOL
Shipping deadline for the main OSD event is Friday 8th of July 2016

1. Take your Sterivex samples out of the freezer for shipping
   a. Leave your sample as long as possible in the freezer (-20°C short term, -80°C long term) so that only the shipping time will be at room temperature

2. **IMPORTANT**: make sure your Sterivex container is correctly labeled with the following information

Correct label for samples for prokaryotes (protocol A)
<OSD_SiteID>_<Month>_<Year>_<SiteName>_<Protocol_Label><SampleNo>_<Depth>
i.e OSD3_06_16_Helgoland_NPL022_1_surface
i.e OSD3_06_16_Helgoland_NE08_1_20m
IMPORTANT: unclear labeling of samples results in rejection!

3. Protect you label from running.
   a. Use a pen that withstands temperature of at least -20°C
   b. For example seal the label on the filter with transparent adhesive tape (also known as Scotch tape, Sellotape or Tesafilm).
   c. Another option would be to use temperature resistant labels (e.g. known as Tough-Tags).

4. Make sure that your filters are packaged in a sterile way.
   a. One option would be to store them in a 50 ml tube and other option is to store them in a small plastic bag. Here are two examples:

5. Please store ALL 4-5 Sterivex samples in a FREEZABLE plastic bag.
   a. Label the plastic bag with the following information
      i. Correct label:
         <OSD_SiteID>_<Month>_<Year>_<SiteName>_<Protocol_Label>_<Depth>
      ii. Contact name & email
      iii. How much water you filtered
      iv. How many filters you places in the plastic bag
   a. It is very IMPORTANT that your samples are stored in a plastic bag that withstands temperature of at least -20°C

b. Protect your label from running by using a pen or a label that withstands temperature of at least -20°C

6. Prepare your samples for shipping
    a. Pack your filters on dry ice. We would recommend to place your filters on 10-20 kg dry ice. IT IS VERY IMPORTANT THAT YOU USE ENOUGH DRY ICE TO GUARANTEE THAT THE SAMPLES ARRIVE FROOZEN (not melted)!
    b. Please send 4-5 Sterivex filters per sampling site. Due to limited storage space at the MPI, we cannot accept more than 4-5 Sterivex filter per sample. Please keep the remaining replicates as backup in your laboratory, preserved in either liquid nitrogen or at -80°C
    c. Filters should be accompanied by a hard copy of the OSD Logsheets (or you can send it in a separate letter) and OSD Data Policy
    d. New OSD sites need also a hard copy of the MTA (Material Transfer Agreement with SI NMNH)
    e. Some sites might also need a signed ABS agreement. Please contact our ABS helpdesk in case of doubt via abs-helpdesk@microb3.eu
    f. Please make sure that you place all hard copies (e.g. logsheets, MTA, Data Policy…) in a sealed plastic bag

7. Some paperwork for the shipping company and custom clearance
    a. Make sure to fill in the Proforma invoice form to specify that your package does not have any content of commercial value - otherwise your samples might not be cleared by customs.
    b. Make sure you fill in any required customs clearance forms - for declaration, please state that the content is "water" so that you will not need to apply for any permits.

8. Dispatch your samples to the Max Planck Institute in Germany (address below)
    a. **IMPORTANT**: make sure your samples preserved in dry ice (you should use at least 15-20kg) arrive in less than 36 hours. If your samples travel longer than 36-48 hours cannot include them in our analysis
    b. **IMPORTANT** FOR INTERNATIONAL SHIPPING: Please dispatch your samples on a Monday (latest on Tuesday) to assure that you samples arrive before the weekend, ideally before Friday.
       Shipping address:
       Max Planck Institute for Marine Microbiology
       Sandra Nowack
       Celsiusstr. 1
       28359 Bremen
       Germany
       Phone:+49 421 2028 – 944
       e-mail: snowack@mpi-bremen.de

9. **SHIPPING DEADLINE:** Please be aware that there is a shipping deadline. Please send your samples to Germany until 8th of July 2016! We cannot guarantee the DNA extraction for any samples arriving after the shipping deadline.

10. Inform us via email that your samples are on their way
    a. Please email <osd-contact@microb3.eu> as soon as you have dispatched your samples
    b. In the "Subject" of your email, please write "<OSD site ID><Site name> shipped: <Date of sample>", i.e. "OSD3 Helgoland shipped: Jun 2016"
    c. Include in your email the following information:
        i. The information you provided in Step 6
        ii. TRACKING NUMBER and name of the shipping company. This way we can track your samples and will be notify upon their arrival.

7.2. Bio-archiving OSD Samples

One replicate of each OSD samples will be cryo-preserved to ensure that the obtained samples can be available in the future as technologies advance.

An agreement for the OSD samples bio-archiving exists with the Smithsonian Institution National Museum of Natural History, USA, which aims to cryo-preserve 50% of the diversity of life in five years and make these collections available for research. The SI NMNH will archive OSD samples for 5 years.

A Material Transfer Agreement between each OSD participant (represented by the OSD Site coordinator) and the SI NMNH has to be signed prior to the OSD campaign and can be found in the **Annex III** of this Handbook.

SI NMNH will also provide for each OSD sample a unique barcode identifier that will be a part of the Sample ID assigned to each sample filter by the OSD Core Team.

OSD sampling stations or cruises shall not send samples to the SI NMNH directly.
OSD sampling stations and cruises shall send their OSD samples to the MPIMM in Bremen (see Chapter 7.1), where the OSD Core Team will further act on their behalf.

SI NMNH Biorepository Pre-shipping Checklist for Ocean Sampling Day

1. The Ocean Sampling Day sample is contained in a Sterivex filter cartridge, which has the ends blocked with sterile, inert material.
2. The OSD Participants' agreement with the Smithsonian Institution, National Museum of Natural History (SI, NMNH) has been signed (it specifies the duration, conditions, responsible parties, and purpose of the transaction; **Annex III**).
3. A **minimum** of 3 business days advanced notice has been given **prior** to shipment (please e-mail NMNHBiorepository@si.edu to arrange a good date for shipping—avoiding holidays, vacations, etc.).
4. All necessary permits (collecting, import/export) are included with the shipment (this provides proof that all samples were collected legally).
5. Include information on the transaction, in writing (gift, loan, or deposit—see "Genomics Research Support" here:
   http://www.mnh.si.edu/rc/biorepository/index.html).
6. Metadata on the sample is provided electronically in a tab or comma-delimited file. Expected data fields include: collector name, collector institution, sample identifying number, sample's country of origin, collection method, date collected (year/month/day), location (using digital WGS84 latitude and longitude format), and depth.*

* The OSD Core Team will prepare a file containing SI NMNH-requested metadata for all OSD samples based on information present in the OSD Site Registry and provided in the OSD Logsheets. OSD sampling Sites do not have to be concerned about this point.

8. Submitting OSD Sample Contextual Data

Samples collected during the OSD Event have a unique value as a global collection representing a single-day snapshot of seas and oceans around the world.
In order to draw meaningful conclusions from data obtained from these samples it is **important that each OSD sample is described with at least the *mandatory OSD checklist***, which is a minimum information placing the OSD sample into its environmental context.

The *mandatory OSD checklistOSD checklist* together with *OSD-recommended information* are described in the Chapter 6 and are included in the OSD Logsheets.

OSD sampling Sites shall upon the OSD sampling event complete the OSD Logsheets (please see the **Annex I**) and **send a copy of the OSD Logsheets together with the OSD samples** to members of the OSD Core Team in the MPIMM in Bremen, Germany (please see the Chapter 7).
The Micro B3 Information System has developed a submission portal for capturing contextual information of the *OSD checklist.*
OSD Sampling Sites shall **transfer ALL information from the hardcopy of the OSD Logsheets into the Micro B3 IS submission portal**.

Please go to the following link and follow instructions on the screen:
http://mb3is.megx.net/osd-registry/sample-registration

Deadline for submission of mandatory metadata is August 14th 2016. Late entry of (optional) metadata can be enabled upon individual request. Please send an email explaining your request to osd-contact@microb3.eu.

Why is it important to submit information to the Micro B3 Information System submission portal and send the OSD Logsheets?

When information from the hardcopy of the OSD Logsheets is transferred to the Micro B3 submission portal, contextual information of the *mandatory OSD checklistOSD checklist* is in the Micro B3 Information System and can be propagated to OSD data holding archives, the PANGAEA and the ENA. Digitalisation of the OSD Logsheets content by submission to the Micro B3 IS submission portal will trigger registration of each OSD sampling Site and OSD samples at the PANGAEA and at the ENA.

OSD sampling Sites do not have to individually register at the OSD data holding repositories since the Micro B3 IS will do so on their behalf. Moreover, a centralised collating of contextual data of all OSD samples via the OSD submission portal significantly simplifies the reporting burden of each OSD sampling Site. Many information pieces otherwise required by the OSD data and metadata holding resources (ENA, PANGAEA, SeaDataNet, EurOBIS) can be inferred from the information provided by OSD sampling Sites in the OSD Logsheets. Furthermore, the early central digitalization of OSD sample contextual data allows a high level of data quality control. The double bookkeeping electronically and on the paper log-sheets allows any of the two to fail while still guarantying successful submission of the OSD data.

9. OSD Environmental and Morphology-based Biodiversity Data

Environmental and morphology-based biodiversity data from the OSD Event will be archived at the PANGAEA, http://www.pangaea.de/.

When OSD sampling Sites digitalise content of their OSD Logsheets by submitting information from the OSD Logsheets into the Micro B3 submission portal, this process will allow registration of each OSD sampling Site in the PANGAEA's client system, creating a separate submission thread for each OSD Site.
Micro B3 IS will transfer the *mandatory OSD checklist* information for each OSD sample to the PANGAEA.

The PANGAEA Team will contact each OSD Site and advise on submission of environmental and morphology-based biodiversity data.

The registration will also allow OSD Sites to update/amend information about their OSD Sampling Event at a later stage, beyond a lifetime of the Micro B3 project.

10. OSD Nucleotide Sequence Data

Molecular data from the OSD Event will be archived at the European Nucleotide Archive at the EMBL-EBI, https://www.ebi.ac.uk/ena/.

When OSD sampling Sites digitalise content of their OSD Logsheets by submitting information from the OSD Logsheets into the OSD submission portal, this process will allow registration of each OSD sample in the ENA data repository, creating for each OSD sample an ENA SAMPLE record with a permanent and unique ENA SAMPLE Identifier. Each ENA SAMPLE record of an OSD sample will contain all attributes of the *mandatory OSD checklist*, ensuring a Micro B3-compliant description of the OSD sample.

Generated nucleotide sequence data from the OSD Campaign will be sent to the MPIMM, where the read data will be demultiplexed and mapped to the ENA SAMPLE Identifiers. The MPIMM Team will then deposit all sequence data to the ENA, where ENA SAMPLE record of each OSD sample will be linked to its corresponding molecular data and made publically available.

The registration of OSD samples at the ENA will also allow OSD Sites to update/amend information about their OSD samples at a later stage, even beyond a lifetime of the Micro B3 project*.

All Micro B3/OSD molecular data can be accessed via the ENA umbrella study PRJEB5129 via the ENA Browser, http://www.ebi.ac.uk/ena/data/view/PRJEB5129**.** Refined search using the *mandatory OSD checklist* attributes is possible via the ENA Advanced Search, http://www.ebi.ac.uk/ena/data/warehouse/search.

* We recommend to update/amend OSD sample contextual data using the ENA submission tool WEBIN, https://www.ebi.ac.uk/ena/submit/sra/#home, designed for small-scale submissions of sequence data and metadata. Please contact datasubs@ebi.ac.uk for any enquiry regarding OSD sample contextual data amendments.

11. OSD Data Access and Analysis

The unique OSD data collection will be integrated into pan-European data resources for open access, discovery and research in the field of molecular microbiology, ecology and beyond. The OSD consortium makes an extensive effort to share all collected OSD data effectively, to propagate relevant pieces of information appropriately to all OSD data and metadata holding centres (PANGAEA, ENA, SeaDataNet, EurOBIS, see the Figure 2) and to allow horizontal communication between these archives as well as vertical communication with the Micro B3 Information System.

The Micro B3 data holding centres will be able to respond to search queries related to data they archive and will refer to information stored in other Micro B3 integrated resources. For instance, the ENA can serve searches for molecular samples within a particular georeference radius and display temperature of water at the time of sampling but for detailed CTD profile a user will be directed to the SeaDataNet, which will collect the relevant data from the PANGAEA, **Figure 5**.

The OSD Information System will be able to serve user queries in a more integrated manner. For instance, molecular data can be displayed in the context of environmental parameters measured at the time of sampling but also in the context of ancillary observations and predictive models, which will be made available via the oceanographic data centres network, **Figure 5**.

The unique OSD data collection provides an excellent opportunity to broaden up our knowledge in marine ecosystems biology. Molecular data from the OSD samples will be analysed via several analysis pipelines, such as the SILVAngs (https://www.arb-silva.de/ngs/) or EBI Metagenomics Portal (https://www.ebi.ac.uk/metagenomics/). Workflows for analysis of OSD-related data have been developed in a collaboration of the Micro B3 consortium with the EU project BioVeL (https://www.biovel.eu).

Further information on OSD data analysis will be available in due course from the OSD Information System.
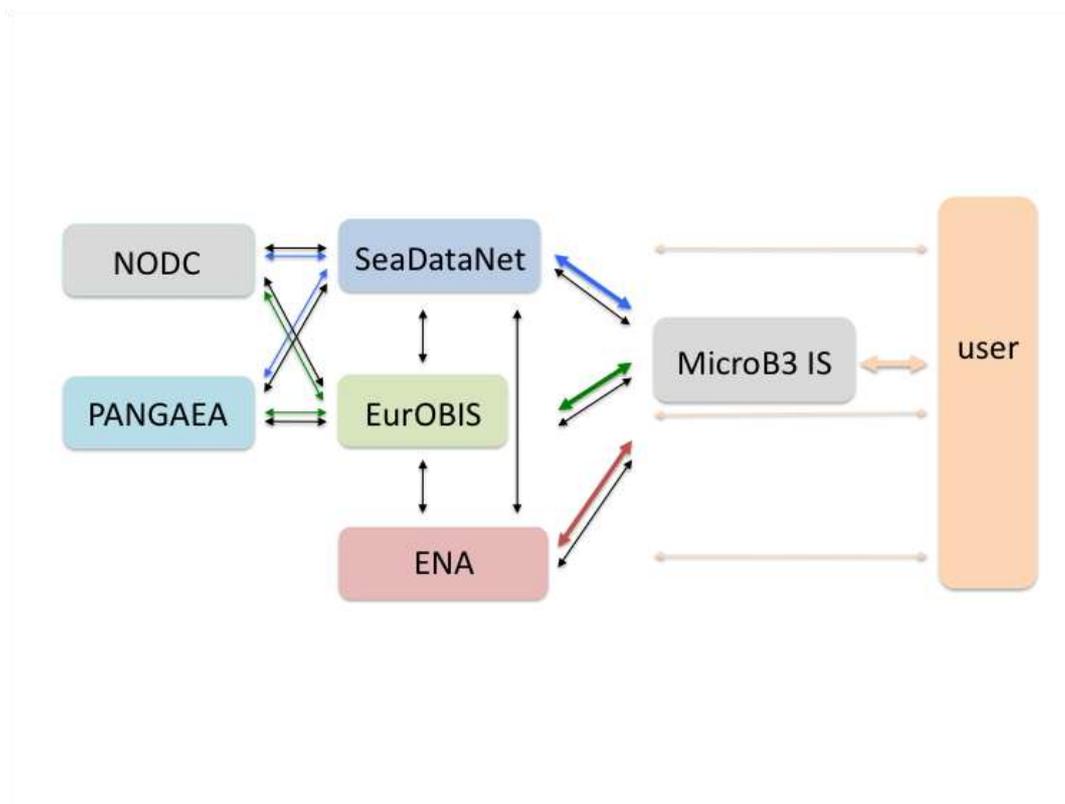
Figure 5: OSD data retrieval workflow. A user can access MicroB3/OSD data directly from individual archives (oceanographic data via the SeaDataNet and Pangaea, biodiversity data via the EurOBIS and molecular data via the ENA). Integrated access to all Micro B3/OSD data will, however, be provided via the OSD Information System that will process a user requests, communicate with the individual data repositories, collect relevant information and serve it back to the user.

Legend: brown full line – material transfer, **black full line** – metadata transfer, blue full line – oceanographic data transfer, **green full line** – biodiversity data transfer, **red full line** – molecular data transfer.

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Not *et al.* (in preparation)

Seth GJ, Mendez CB, Deng L, Poulos B, Kauffman AKM, Kern S, Brum J, Polz MF, Boyle EA, Sullivan MB (2011) A simple and efficient method for concentration of ocean viruses by chemical flocculation. Environmental Microbiology Reports 3:195-202

Annex I
OSD Logsheets

<table>
<tr><td rowspan="8">SAMPLING</td><td colspan="2"></td><td colspan="2">SAMPLING_Site (ID, Name):</td><td colspan="4"></td></tr>
</table>

SAMPLING_Site (ID, Name):			
SAMPLING_Platform:			
SAMPLING_Campaign:			
SAMPLING_Project :	OSD2016		

SAMPLING_Investigators :	Last name	First name	email

SAMPLING_Objective	

EVENT

EVENT_DateTime (UTC)		yyyy	mm	dd	hh	mm
	Start					
	End					

EVENT_Lat/Long		+N/ -S	dd.0000	+E/-W	ddd.0000
	Start				
	End				

EVENT_Device :	
EVENT_Method :	
EVENT_Comment :	

ENVIRONMENTAL SAMPLE

ENVIRONMENT_ Marine_Region	e.g. Adriatic Sea	
ENVIRONMENT_Depth (m): & free text description:		
ENVIRONMENT_ Biome	e.g. ENVO:00000447 for "marine biome"	
ENVIRONMENT_ Feature	e.g. ENVO:00002042 for "surface water"	
ENVIRONMENT_ Material	e.g. ENVO:00002010 for "seawater"	
ENVIRONMENT_ Temperature (deg C)		

ENVIRONMENT_ Salinity			

| EVENT | OSD | EVENT_DateTime_Start : | | | | | |
| | | ENVIRONMENT_Depth (m) : | | | | | |

	Replicates:	Filter #1	Filter #2	Filter #3	Filter #4	Filter #5	Filter #6
FILTER SAMPLE	**SAMPLE_Title:**						
	SAMPLE_Protocol_Label :						
	SAMPLE_Quantity :						
	SAMPLE_Filtration_Time :						
	SAMPLE_Container :						
	SAMPLE_Content :						
	SAMPLE_Size-Fraction_ Upper-Threshold :						
	SAMPLE_Size-Fraction_ Lower-Threshold :						
	SAMPLE_Treatment_ Chemical :						
	SAMPLE_Treatment_ Storage :						

| EVENT | OSD Ocean Sampling Day | EVENT_DateTime_Start : | |
| | | ENVIRONMENT_Depth (m) : | |

	CATEGORY	PARAMETER	DESCRIPTION	VALUE
ENVIRONMENTAL	CTD	Conductivity	Electrical conductivity of water	
		Temperature	**Temperature of water**	
		Depth (m)	Vertical spatial coordinates	
		Salinity	**Salinity of water**	
		Fluorescence	Raw (volts) or converted (mg Chla/m^3) fluorescence of the water	
	Seawater Nutrients Concentration	Nitrate	Nitrate concentration parameters in the water column	
		Nitrite	Nitrite concentration parameters in the water column	
		Phosphate	Phosphate concentration parameters in the water column	
		Silicate	Silicate concentration parameters in the water column	
		Ammonium	Ammonium concentration parameters in the water column	
	Seawater Chemical Properties	pH	Alkalinity, acidity and pH of the water column	
		Dissolved oxygen concentration	Dissolved oxygen parameters in the water column	
	Seawater Optical Properties	Downward PAR	Visible waveband radiance and irradiance measurements in the water column	
		Turbidity	Transmittance and attenuance of the water column	
	Organic Matter Concentration (Amount or Mass)	Carbon organic particulate (POC)	Particulate organic carbon concentration in the water column	
		Nitrogen organic particulate (PON)	Particulate organic nitrogen concentration in the water column	
		Carbon organic dissolved (DOC)	Dissolved organic carbon concentration in the water column	
		Nitrogen organic dissolved (DON)	Dissolved organic nitrogen concentration in the water column	

EVENT	OSD	EVENT_DateTime_Start :	
	Ocean Sampling Day	ENVIRONMENT_Depth (m) :	

	CATEGORY	PARAMETER	DESCRIPTION	CHECK if done
ENVIRONMENTAL	Organism Concentration (Amount, Volume or Mass)	Pigment concentrations	Concentration of pigments (e.g. chlorophyll a) extracted and analysed by fluorometry or HPLC	
		Picoplankton (Flow Cytometry)	Abundance of cells in the water column (+other avail. cell properties)	
		Nano/Microplankton	Abundance of cells in the water column (+other avail. cell properties)	
		Meso/Macroplankton	Abundance of individuals in the water column (+other avail. properties)	
	Community Production Rate	Primary Production (isotope uptake)	Primary Production in the water column	
		Primary Production (oxygen)	Primary Production in the water column	
		Bacterial production (isotope uptake)	Bacterial production in the water column	
		Bacterial production (respiration)	Bacterial production in the water column	

63

			64	

Annex II

Agreement on the Transfer of Marine Microorganisms and Metadata from an OSD Sampling Institution to the Max Planck Institute for Marine Microbiology (MPIMM)

THIS AGREEMENT is made on _____ [insert date]
BETWEEN:
OSD sampling Institution

_____

[Insert the name of the OSD sampling Institution and its representative and the full contact details]

("the Transferor")

AND:
Max Planck Institute for Marine Microbiology

_____

 [Insert the name of MPIMM representative and the full contact details]

("the Transferee")
hereinafter referred to as "the Parties".

PREAMBLE

This Agreement shall contribute to the Micro B3 Project by framing the transfer from a OSD sampling Institution to the Max Planck Institute for Marine Microbiology of samples of marine microorganisms and metadata accessed in the framework of the Micro B3 project. It shall ensure that the Max Planck Institute for Marine Microbiology endorses the Micro B3 Agreement on Access to Marine Microorganisms and Benefit Sharing or any other agreement or permit with similar content concluded between the OSD sampling Institution and a Provider State, as appropriate.

The Parties to this agreement hereby agree as follows:

Article 1 TRANSFER OF GENETIC RESOURCES

1.1 The Transferor will deliver to the Transferee samples of marine microorganisms accessed in the framework of the Micro B3 project.

   a.) Kinds of samples _____

   b.) Number and quantity of samples_____

   c.) Time period of delivery _____

   d.) Form of delivery_____

1.2 The Transferor will deliver to the Transferee all mandatory Micro B3 metadata according to the checklist.

Article 2 VIRAL CLAUSE

2.1 The Transferee endorses the provisions specified in article 5.1 of the Micro B3 Agreement on Access to Marine Microorganisms and Benefit Sharing or the relevant provision in any other agreement of similar content concluded between the Transferor and the competent national authority of …. as indicated by its National Focal Point to the CBD (the provider of the genetic resources in the original agreement).

2.2 The said agreement is attached to the present agreement.

(Location, Date)

(Transferor)   (Transferee)

Annex III

Material Transfer Agreement
Between
Ocean Sampling Day (OSD) Participant [FILL IN] and
Smithsonian Institution National Museum of Natural History

I. Introduction

This agreement, effective as of the date of the last approving signature on this Agreement, between OSD Participant **[FILL IN]** and the Smithsonian Institution National Museum of Natural History (SI NMNH), conveys to the SI NMNH custodial responsibilities as more fully set forth herein, while retaining for OSD Participant **[FILL IN]** ownership responsibilities for up to 10,000 Ocean Sampling Day environmental samples and associated metadata ("OSD Collections") including those covered by this Agreement.

II. Objectives

A. To cold preserve up to 10,000 environmental samples collected through OSD collecting events leading up to June 21, 2014. If OSD continues beyond the 2014 event and the maximum number of 10,000 samples has not been deposited, SI NMNH will continue to accept samples until the maximum number of 10,000 samples is reached or until June 21 2019, whichever comes first.

To enable the loan of the Collections to the SI for a period of six years, beginning on June 21 2013 and ending on June 21 2019. OSD samples will be preserved in a "time-capsule" fashion, meaning that there will be no access to the physical collection during this period, ending after five years on June 21 2018.

III. Scope

This Agreement covers Collections and associated metadata from OSD Participant **[FILL IN]** provided to SI NMNH for custodial purposes on or after the Effective Date of this Agreement. The OSD Collections are limited to a total of 10,000 environmental samples. The total 10,000 environmental samples will be sent in portions to the SI NMNH Biorepository by the OSD Participants and/or from the Jacobs University Bremen. The number of samples included within each portion sent to the SI NMNH Biorepository will be determined by OSD Participants and/or from the Jacobs University Bremen.

IV. **Terms**

SI NMNH will:

A. Cold preserve up to 10,000 environmental samples at -80°C collected on or after June 21 2014 and OSD pilot collecting events leading up to June 21 2014, provided they comply with the OSD Bioarchiving pre-shipping checklist and meet the SI NMNH data standards. Expose, for the duration of the loan and as its systems will allow, information on basic data on OSD samples to be preserved, including: collector name, collector institution, sample identifying number, sample's country of origin, collection method, date collected (year/month/day), location (using digital WGS84 latitude and longitude format), and depth.

B. Respect the conditions of the Access and Benefit–sharing Agreement (under MicroB3) and/or any other permit document according to which access to the samples has been granted from the Provider country.

C. Provide care for the OSD samples to the same standard as is used for NMNH-owned samples, with the provision that neither the NMNH nor Smithsonian Institution can be held liable for loss in the event of catastrophic facility or equipment failure.

OSD Participant **[FILL IN]** will:

A. Agree to follow the SI NMNH Biorepository Standards and Services statement, http://www.mnh.si.edu/rc/biorepository/standards_services.html.
B. Agree to follow the OSD Bioarchiving pre-shipping checklist.
C. Agree to cover the cost of shipping and handling.
D. Provide SI NMNH with all permits related to collecting and transporting the OSD Collections.
E. Provide SI NMNH with a copy of the Access and Benefit–sharing Agreement (within OSD Consortium) and/or any other permit document according to which access to the samples has been granted from the Provider country and containing constraints for the use of the samples.
F. Abide by all applicable laws and regulations, including appropriate access and benefits sharing agreements and/or any other permit document as illustrated in E. above.
G. Provide SI NMNH with associated metadata compliant with the SI NMNH data standards.
H. Apply labels to filters as specified by NMNH.

V.   **Period of agreement**

This agreement and SI NMNH's custodial responsibilities shall remain in effect for a period of six years starting June 21 2013 and ending on June 21 2019. This agreement will become effective when signed by both parties. At the close of this agreement OSD participants, provider countries (the country supplying genetic resources collected in situ), and SI NMNH will re-negotiate this present agreement and the need for SI NMNH to retain custodial responsibilities of the OSD collection.

VI.   **Agreed**

OSD Participant [1]   SI NMNH
By   By
Contact name   Contact name
OSD Site ID

---

[1] The signature should be one of a legal representative of the OSD participant institution or a person entitled to sign on its behalf.

Annex IV

Micro B3 Model Agreement on Access to Marine Microorganisms and Benefit Sharing

Result of Micro B3 WP8
http://www.microb3.eu/
17th December 2013

Authors: Caroline von KRIES(1), Arianna BROGGIATO(2), Tom DEDEURWAERDERE(2), Gerd WINTER(1).
(1) Universität Bremen, Germany
(2) Université catholique de Louvain, Belgium
Please address correspondence and comments to tom.dedeurwaerdere@uclouvain.be

Agreement on Access to Marine Microorganisms and Benefit-Sharing

THIS AGREEMENT is made
BETWEEN:

_____
[Insert the name of the Provider State institution[2] and its representative and the full contact
 details]

("the Provider")

AND:

_____
 [Insert the name of the Recipient institution[3] and its representative and the full contact
 details]

("the Recipient")

hereinafter referred to as "the Parties".

PREAMBLE

Considering that the European Union funded research project Micro B3 (hereinafter the
 "Micro B3 Project") is a scientific research program with the following objectives:

- to cooperatively sample marine microbial biodiversity at various sites, including
  through global coordinated actions called "Ocean Sampling Days"
- to generate large-scale knowledge on marine microbial genomes in an
  environmental context and on actual or potential biotechnological applications
- to develop innovative bioinformatics approaches for the large scale integration of
  genomic data of marine microbes with environmental and ecosystems data
- to make the resulting knowledge accessible for the research and development
  community for policy makers and the public at large,

Recalling that access to and utilization of genetic resources taken from the marine internal
 waters, territorial sea, exclusive economic zone or continental shelf of coastal states
 should be consistent with the provisions of the Convention on Biological Diversity (CBD)
 taking into account their specifications by the Bonn Guidelines on Access to Genetic
 Resources and the Fair and Equitable Sharing of Benefits arising from their Utilization,
 and, where appropriate, the Nagoya Protocol on Access to Genetic Resources and the
 Fair and Equitable Sharing of Benefits arising from their Utilization (NP, not yet in force),
 as well as with the United Nations Convention on the Law of the Sea (UNCLOS) and the
 customary law expressed by UNCLOS,

---

[2] The Provider must be empowered to represent the Provider State concerning the granting of a permit and the conclusion
of an agreement on access to marine genetic resources, the utilization of genetic resources, the transfer of genetic
resources and knowledge and the sharing of benefits drawn from its use.

[3] The Recipient shall not be the individual researcher but the institution which employs the researcher. This ensures that
the agreement survives changes of personnel and that its implementation is surveyed.

Recalling that according to these provisions access to and utilization of genetic resources taken from the above described maritime zones is subject to prior informed consent of the coastal state and mutually agreed terms if the coastal state so requires,

Recalling that according to these provisions coastal states have the right to regulate, authorize and conduct marine scientific research in their marine internal waters, territorial sea, exclusive economic zone and on their continental shelf; and that in the case of research undertaken by other states or international organizations the coastal state has the right, if it so desires and if practicable, to participate or be represented in the marine scientific research project and to access data and samples and receive preliminary reports, and final results,

Recalling that according to these provisions non-monetary and/or monetary benefits from the utilization of the genetic resources shall be shared with the Provider State if the same so requires and as it is set out in mutually agreed terms,

Recalling that according to these provisions the transfer of genetic resources to third parties shall be set out in a material transfer agreement,

Recalling that according to these provisions measures on access for non-commercial research purposes shall be simplified with a view to contribute to the conservation and sustainable use of biodiversity, and

Acknowledging that research and development on genetic resources can be for the public domain or for proprietary purposes,

The Parties to this agreement hereby agree as follows:

Article 1 AGREEMENT

1.1 The agreement sets out the terms for the access to genetic resources found in/on the Provider State's marine internal waters, territorial sea, exclusive economic zone or continental shelf, for the utilization and transfer to third parties of the accessed genetic resources, for the management and transfer to third parties of associated knowledge and for the sharing of benefits drawn from the same.

1.2 The agreement is part of the Micro B3 Consortium Agreement[4]. Its rights and obligations extend to all Micro B3 partners.

1.3 The Parties agree to release a copy of the agreement to the registered users of the web portal built by the Micro B3 project.

Article 2 DEFINITIONS OF TERMS

As used in this agreement, the following terms shall have the meaning provided below:

a) **Access** means collecting genetic resources from the location where they are found.

b) **Accessed genetic resources** means the genetic resources collected on the basis of this agreement.

---

[4] The Consortium Agreement is publicly accessible at the Micro B3 website www.microb3.eu

c) **Associated genetic knowledge** means any experimental or observational data, information and other findings on the composition, life conditions and functions of the accessed genetic resources.

d) **Derivative** means a naturally occurring biochemical compound resulting from the genetic expression or metabolism of biological or genetic resources, even if it does not contain functional units of heredity.

e) **Genetic resources** means any material of plant, animal, microbial or other origin containing functional units of heredity which is of actual or potential value.

f) **Micro B3 partner** means an institution that is a Party to the Micro B3 Consortium Agreement.

g) **Ocean Sampling Days** are simultaneous sampling campaigns in the world's oceans, as part of the Micro B3 project, aiming at providing insights about the microbial diversity and the identification of novel ocean-derived biotechnologies.

h) **Provider State** means the coastal state from whose marine internal waters, territorial sea, exclusive economic zone or continental shelf genetic resources are collected *in situ*.

i) **Third party** means any institution other than Micro B3 partners.

j) **Utilization for proprietary purposes** means research and development that aims at protecting the associated knowledge, including products and processes developed, by patent rights, keeping the associated knowledge secret, making the associated knowledge accessible at more than incremental costs for dissemination and/or bringing the products and processes developed from the accessed genetic resources on the market.

k) **Utilization for the public domain** means research and development that aims at making the associated knowledge, including products and processes developed, publicly available at no more than incremental costs for dissemination, and without being protected by patent rights or further restricted by other intellectual property rights.

l) **Utilization of genetic resources** means research and development on the genetic and/or biochemical composition of the accessed genetic resources, including through the application of biotechnology which is any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Article 3 ACCESS TO GENETIC RESOURCES

3.1 The Recipient shall be entitled to collect samples as follows:

a) Kinds of samples[5], including the kind of genetic resources[6], if known:

_____

_____

b)   Number   and   quantity   of   samples:
_____

c)   Geographical   location   of   collection[7]:
_____

d)   Time   period   for   collection:
_____

3.2 The Recipient shall within … [time period to be specified by the Parties] after collection of the samples notify to the Provider the kinds of genetic resources the Recipient intends to utilize. The Provider may, within … weeks [to be specified], raise objections in which case the Parties will seek agreement on the kinds of genetic resources allowed to be utilized.
(This clause is to be crossed out if not applicable)[8]

3.3 The Recipient shall be entitled to move the accessed genetic resources to its premises and, subject to Article 1.2 of this agreement, to the premises of other Micro B3 partners, as well as to an institution or individual which is contractually bound with the Recipient to provide specified assistance concerning the utilization of the accessed genetic resources[9].

3.4 The Recipient shall deliver a portion of the accessed genetic resources to the Provider or an institution designated by the same:

_____

_____
The samples shall be delivered in the following form:
_____-
_____
(This clause or part of it is to be crossed out if not applicable)

3.5 The Recipient shall bear all the costs incurred in accessing and delivering the genetic resources.

Article 4 UTILIZATION OF THE GENETIC RESOURCES

---

[5] E.g. seawater, sediment.

[6] The kind of genetic resources to be extracted from the sample, i.e. virus, bacteria, funghi, microorganism.

[7] E.g. GPS coordinates.

[8] Not applicable if kind of genetic resources included is known *ex ante* under 3.1.a)

[9] All other transfers are considered transfers to third parties and bound by the conditions under Article 5.

4.1. The Recipient shall be entitled to the utilization of the accessed genetic resources.
Specifications, if deemed necessary:
_____
_____
_____

4.2 The utilization of the accessed genetic resources shall be for the public domain.
Specifications, if deemed necessary:

_____
_____
_____
_____
(This clause is to be crossed out if not applicable)

4.3 The Recipient shall be entitled to utilize part/all (please cross out) of the accessed
genetic resources for proprietary purposes:
Specifications, if deemed necessary:
_____
_____
_____
(This clause is to be crossed out if not applicable)

4.4 Should the Recipient, after the conclusion of this agreement, intend to utilize the
accessed genetic resources and/or use the associated genetic knowledge for
proprietary purposes the Recipient shall seek the consent of the Provider.
Specifications of the consent procedure, if deemed necessary:
_____
_____
_____
_____

4.5 Should the Provider, after the conclusion of this agreement, intend to utilize the
accessed genetic resources and/or use the associated genetic knowledge for proprietary
purposes the Provider shall enter into amicable negotiations with the Recipient on the
modification or termination of this agreement.
(This clause is to be crossed out if not applicable)

Article 5 TRANSFER OF GENETIC RESOURCES TO THIRD PARTIES

5.1 The Recipient may transfer to a third party the accessed genetic resources, or parts of
them, provided that the third party agrees with the Recipient, to apply to the
transferred genetic resources Articles 4 to 16 of this agreement.

5.2 If the Recipient intends to transfer to a third party the associated genetic knowledge
which is not yet submitted to the public domain according to Article 6, the third party
shall agree with the Recipient, to apply to the transferred knowledge Articles 4 to 16 of
this agreement.

5.3 In case of transfer to a third party, the Recipient needs the prior informed consent of the

Provider, under one of the following modalities:[10]

- a notification of the transfer to the Provider or an institution designated by the same, along with the sending of a copy of the transfer agreement, will be considered as proof of prior informed consent. The institution shall be the following [if applicable]:

_____

_____

- other [specification of the modality]:

_____

_____

[This clause is to be crossed out upon agreement that the consent is not required]

Article 6 DISSEMINATION OF KNOWLEDGE

6.1 The Recipient shall make the associated genetic knowledge publicly available at no more than incremental costs of dissemination. The dissemination can be through online media, print media or delivery upon request. The recommended forums for online dissemination are the Micro B3 Information System (www.microb3.eu) and existing data bases and information networks such as the Global Biodiversity Information Facility (GBIF), SeaDataNet, Pangaea and the International Nucleotide Sequence Database Collaboration (INSDC).

6.2 Such knowledge shall be made available as soon as possible after its generation unless otherwise specified. No embargo period is allowed for the raw sequence data and the oceanographic data associated to the samples collected upon the Ocean Sample Days. Specifications if deemed necessary:_____

_____

_____

6.3 The Recipient shall make reasonable efforts to ensure that the release of associated genetic knowledge through online media, print media or delivery upon request will be organized such that users are bound not to use the associated genetic knowledge taken from the portals for proprietary purposes unless they have obtained prior informed consent of the Provider.

---

[10] NOTE OF CAUTION: The Parties should be aware that too heavy PIC requirements could significantly complicate the research and development process during the non-commercial stage considered in this contract (defined as public domain). A facilitated PIC procedure for non-commercial use (public domain uses) as proposed here would also be to the advantage of the Provider country because this allows the Recipient to transfer GR or knowledge during the non–commercial stages more easily and this might lead to increased commercial product development in later stages, in which a new negotiation with the Provider country is initiated according to the renegotiation clause in Art. 4.4.

6.4 Paragraphs 1-3 of this Article do not apply to associated genetic knowledge used for proprietary purposes specified under Articles 4.3 and 4.4.

6.5 The Recipient shall make reasonable efforts to ensure that the users of knowledge accessed from the Micro B3 Information System provide to the System the knowledge from their own research in such form and format as the System will reasonably require in order to promote the objectives of the utilization for the public domain.

Article 7 ACKNOWLEDGING THE CONTRIBUTION OF THE PROVIDER STATE

7.1 When making associated genetic knowledge publicly available under Article 6 the Recipient shall indicate the country of origin of the utilized genetic resource.

7.2 When making associated genetic knowledge publicly available under Article 6 the Recipient shall acknowledge the role of scientists from the Provider State, and, where any work, significant advice or recommendations have been provided by such scientists, their (co-)authorship.

Article 8 RECORDING AND REPORTING

8.1 The Recipient shall maintain records concerning the storage and transfer of the accessed genetic resources and allow access to such records to the Provider or the authority designated by the same.

_____
_____
_____ (insert name and address of authority if applicable)

8.2 The Recipient shall report in writing to the Provider or the authority designated by the same every _____ [insert duration] months, beginning _____ and ending _____, providing details of the progress of utilization.

_____
_____
_____(insert name and address of authority if applicable)

8.3 With relation to associated genetic knowledge used for proprietary purposes specified under Articles 4.3 and 4.4, the Recipient shall, when reporting according to paragraph 2 of this Article, also report on any steps taken towards obtaining or implementing intellectual property protection and the selling of products or processes based on this knowledge[11].

Article 9 SHARING OF KNOWLEDGE

---

[11] Subject to negotiation of the Parties it could be agreed that the consent of the Provider is required for certain steps of commercialization such as the bringing on the market of the product.

9.1 The Recipient shall provide the Provider, or the authority designated by the same, with the associated genetic knowledge and provide assistance in their assessment or interpretation as reasonably requested.

_____
_____

_____ (insert name and address of authority if applicable)

9.2 Such knowledge shall, at the latest, be provided once it has been made publicly available.
Specifications   if   deemed
necessary[12]:_____

_____
_____

9.3 The obligation under paragraph 1 of this Article extends to associated genetic knowledge used for proprietary purposes specified under Articles 4.3 and 4.4. When using the knowledge the Provider shall not prejudice any use for proprietary purposes by the Recipient. [13]
Specifications,   if   deemed   necessary:
_____
_____
_____
(This clause is to be crossed out if not applicable)

9.4 The Recipient shall furnish the Provider or the authority designated by the same with _____ (insert number) copies of any publication based on the utilization of the accessed genetic resources.

_____
_____

_____ (insert name and address of authority if applicable)

Article 10 SCIENTIFIC COLLABORATION WITH THE PROVIDER STATE AND CAPACITY-BUILDING

As part of the Micro B3 project the Recipient agrees to collaborate with scientists from the Provider State in the utilization activities based on this agreement. Such involvement shall take the following forms:

_____
_____

---

[12] It may be concluded between the Parties that the Provider shall be informed before publication. This may allow the Provider to check if the requirements under Article 7 are fulfilled and/or if there is reason for pursuing proprietary purposes according to Article 4.5. In this case the provider shall keep the knowledge confidential during the agreed period.

[13] This clause will be negotiated along with the benefit-sharing arrangement: a provider country will prefer to have access to the information (even if the country keeps it confidential as specified under 9.2), but a company might prefer to give a higher upfront benefit-sharing under Article 11 as a *quid pro quo* for crossing this Article.

_____
                                    14
_____
(to be specified by negotiations)

Article 11 BENEFIT-SHARING IN CASE OF UTILIZATION FOR PROPRIETARY PURPOSES

11.1 The Recipient agrees to pay an up-front compensation of … (amount to be specified) to the Provider, if the Recipient utilizes the accessed genetic resources for proprietary purposes. The payment is due to the Provider within … months (term to be specified) after consent on the kinds of genetic resources to be utilized has been reached under Article 3.2. The payment shall be transferred to the following account of the Provider:

_____
_____
(This clause is to be crossed out if not applicable)

11.2 If the Recipient utilizes the accessed genetic resources or uses the associated knowledge for proprietary purposes according to Articles 4.3 and 4.4, it must fairly and equitably share with the Provider any monetary benefit obtained.

11.3 The share shall be determined by further negotiations between the Parties to this agreement.

11.4. (Alternatively to 11.3) The share shall be _____percent of the revenue from sales of the product or process based on the accessed genetic resources. It shall be paid on the basis of a financial report to be sent to the Provider or an authority designated by the same at the end of any year of any revenue generation to the account designated by the same.

_____
_____
_____
_____
(Insert authority and account details if applicable)

11.5 If the Recipient utilizes the accessed genetic resources or utilizes the associated genetic knowledge for proprietary purposes without being entitled according to Articles 4.3 or 4.4, and therefore in breach of the conditions of this agreement, it must share with the Provider any monetary benefit obtained from such utilization or use. The share shall be _____ percent of the revenue from sales of the product or process based on the accessed genetic resources. It shall be paid on the basis of a financial report to be sent to the Provider or an authority designated by the same in due time upon request by the same.

_____
_____

_____
[14] It should be noted that in the normal case of scientific collaboration the partners conclude a research collaboration contract in which the details of the collaboration are laid out. The ABS agreement should not be overloaded with such details. It will be advisable that the Parties to the ABS agreement make a reference to the research collaboration agreement.

_____

_____

(Insert authority and account details if applicable)

(This Article or single paragraphs of it are to be crossed out if not applicable)

Article 12 OTHER LAWS TO BE RESPECTED

The Recipient shall ensure that the collection, storage, transfer, utilization and exportation of the genetic resources complies with all applicable laws of the Provider State on the protection of human health and the environment, on taxes, on customs and any other concern.

Article 13 DURATION OF THE AGREEMENT

The agreement is of unlimited duration, except for the obligations under Articles 8.2 and 10 which shall end on [date to be inserted; e.g. 2 years after the termination of the Micro   B3 project]:_____ _____

Article 14 APPLICABLE LAW

14.1 The applicable law on any matters relating to the interpretation and the application of the present agreement shall be:

_____
_____

14.2 The competent court for dispute settlement shall be:

_____
_____

Article 15 DISPUTE SETTLEMENT

15.1 No Party shall, in the event of a dispute arising from this agreement, commence court proceedings (except proceedings for urgent interlocutory relief) before searching for an amicable solution according to paragraphs 2 and 3 of this Article.

15.2 A Party to this agreement claiming that a dispute has arisen under or in relation to this agreement must serve the other Party with a written notice specifying the nature of the dispute on receipt of which the dispute resolution shall forthwith begin.

15.3 Any dispute arising from this agreement shall be resolved expeditiously foremost by negotiation in good faith failure to which the Parties shall engage informal dispute resolution techniques, such as mediation and arbitration or similar techniques agreed to by them.

Article 16 TERMINATION OF THE AGREEMENT

16.1 The agreement may be terminated at any time by mutual agreement in writing.

16.2 The agreement may be terminated by default if the Recipient fails to satisfy any of the following obligations under this agreement: Articles 4.2, 4.3, 4.4, 5.1, 5.2, 5.3, 6.1, 6.3, 7, 8, 9.1 and 9.3, 11.2 and 11.5.

16.3 In the case of default the Provider may immediately terminate this agreement by giving written notice to the Recipient of the termination, provided that:

   a) the Provider has given prior notice to the Recipient of the alleged default; and

   b) the Recipient fails to respond to the Provider within the period specified by the notice (being not less than 20 business days and not more than 60 business days) to rectify or explain to the satisfaction of the Provider the reasons for the default.

16.4 If this agreement is terminated under paragraph 2 of this Article the Recipient will not thereafter utilize or transfer the accessed genetic resources or use or transfer associated genetic knowledge; and it will transfer back to the Provider or destroy, at the Provider's discretion, all genetic resources or associated genetic knowledge. The operation of this clause survives the termination of this agreement.

(Location, Date)

(Provider)   (Recipient)

Annex V

OSD Data Policy

Updated 3.05.2016

Authored by the OSD Data Policy Working Group with the OSD Consortium

OSD Data Policy Working Group

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Status to date

This data policy covers the collection, dissemination, analysis and publication of OSD data. It was developed over the past years of OSD pilot events. Within Micro B3, WP8 was responsible for the Micro B3 data policy. The OSD policy is based on this policy and consultation with the OSD Consortium. It was finalized at the Micro B3 General Assembly (GA) April 2014. It remains the responsibility of the OSD Data Policy working group to make sure the policy is fit for purpose and all are aware of it when submitting and using data of the OSD Consortium. If you would like to join the group please contact osd-contact@microb3.eu.We welcome input from all OSD participants on shaping of the OSD data policy. Please send comments to osd-contact@microb3.eu.

Background

The OSD Data Policy was created by drawing on existing policies (See Biosharing: http://biosharing.org/policies) and current best practice in genomics and the needs of Micro B3 and the OSD community. It was developed and will be maintained according to the "12 steps to a data policy" outlined in *Field et al (2009) Science:*

12 Step Path to a Data Policy:
http://blog.biosharing.org/2010/02/12-steps-to-creating-data-sharing.html

Tenets of Ocean Sampling Day (The policy applies to all in the OSD Consortium. It is a mandatory part of taking part in OSD)

The OSD data set is generated by the members of the OSD Consortium - to be part of the Consortium you must be formally registered and substantively contribute to the collaboration.

The OSD dataset will be a reference data set and should be as widely accessible and used to support downstream research as possible.

As OSD is one event (a one-off event) it should be easier to get all contributors (their official sites) to release the data publicly.

All OSD data must be standards compliant - must include metadata - this includes compliance with the Micro B3 standard, which includes the GSC's MIxS standard.

All OSD genomic data must be tightly linked to environmental data - contextual information is a top priority.

Sequence data without deposition of accompanying metadata (minimum of lat, long, time, depth, temp, salinity according to OSD Handbook) will not be released after the June solstice event. The OSD Consortium will work towards release of all data.

The OSD data policy covers the pilot events, the main OSD event and any subsequent events.

Data must be submitted to the public repositories (e.g. INSDC). The OSD Team will make sure 2012-2016 data sets are submitted to the ENA/MG-Portal.
All data will be processed/stored in the OSD infrastructure as it is built and the full data set will eventually be available through this infrastructure. Data can flow in many analysis pipelines/databases - we welcome its wider use

All samples must be collected legally (with appropriate permits) and individual collectors are responsible for making sure of this. Specifically ABS/MTA/DTA agreements will be in place as required (bioarchiving at GGI requires OSD MTA, proof of relevant ABS and metadata)

The OSD Consortium

This data policy applies to everyone in the OSD Consortium (to date this means everyone who has constructively contributed to OSD sampling events).

Release of the data and metadata

At the 2013 EEB meeting of the Micro B3 project we agree on the importance of the OSD data set as a whole (the sum of participating sites) and the desire to support open sharing of the data. The EEB agreed to work towards: "immediate release of all OSD data as soon as sequenced and quality checked to the public". In this, sense "public" means beyond Micro B3 and OSD participants to the public at large. To protect the rights of the consortium to publish the first global analysis of the data, the data will be released under Ft Lauderdale principles. This is routinely used in large

sequencing projects and promotes the use of the data by the wider community while safeguarding the scientific contributions of the data generators.

This statement will accompany all access to OSD sequence data.

Use of the sequence data

All data will be made freely available. However, we ask users to observe the Ft. Lauderdale principles, which entitles the data producers to make the first presentation and publish the first genome-wide analysis of the data. The data can be used freely for studies of individual genes or other individual features of these sequences.

Collaborative Data Analysis

We hope that the OSD reference data set will be helpful in a variety of ways and we encourage those interested in analysis of the data to declare their interests and formally join the OSD Data Analysis working group. Together, we are working towards "OSD reports" that will be generated following each event. Ideally, each report will provide a high level overview of the event and, if multiple events are available, comparison and contextualization with past data. Predictions about future events may also be included. It is our vision that this type of transparent collaboration where interested parties declare how they would like to contribute to the data analysis, will help maximize the efforts of the community and build the strongest possible biological interpretation of the data.

Publication

We expect the authorship of the global analysis of the OSD data set to belong to the OSD Consortium. Authorship will be defined on a case-by-case basis for more specialist publications.

Evolution of this Policy

According to the "12 steps for policy development" process, this policy may evolve according to best practice. As agreed at the May 2013 EEB meeting of Micro B3, changes to the policy will be done in consultation with the community. Specifically, policy issues will be taken forward through the OSD Data Policy Working group who is responsible for building consensus on any issues or changes. Final authority for any changes and for upholding the policy will rest with the OSD Team.

Signed by

Site ID: …………………………………………………...........

Site Name: …………………………………………………......

Institution: …………………………………………………......

Country: …………………………………………………......

Site Coordinator: …………………………………………………......

Signature: ....

Date: …………………………………………………..