



Marine Microbial Biodiversity, Genomics & Biotechnology



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Ocean Sampling Day Handbook

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Generalist Summary

The Ocean Sampling Day (OSD) Handbook, version 1.0, 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 main sampling event of the Micro B3 project, the Ocean Sampling Day 2014, (<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 standardized protocols and accompanied by a standardized 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 in PANGAEA (<http://www.pangaea.de>), condensed summary of oceanographic data in SeaDataNet (<http://www.seadatanet.org/>) and morphology-based biodiversity data in EurOBIS (<http://www.marbef.org/data/eurobis.php>). OSD sample metadata, contextual data 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.

Summary

The Micro B3 work package 4 concerns the development of interoperability structures and standards for marine sampling that will support better integration and usability of acquired marine data. Deliverable D4.3, the Ocean Sampling Day (OSD) Handbook version 1.0, builds on results reported in D4.1 and D4.2, and on efforts of Micro B3 work packages 2, 3, 5 and 8. It summarises best practice on marine sample and data collection, and marine metadata reporting for participants of the principle Micro B3 sampling campaign – Ocean Sampling Day. The OSD Handbook also provides essential guidelines for submission of environmental and molecular OSD data. In order to incorporate ongoing development in the Micro B3 and at the same time offer the best possible support for the OSD event in June 2014 the OSD Handbook will have incremental versions.

The Ocean Sampling Day Handbook, version 1.0, is available at http://www.microb3.eu/sites/default/files/deliverables/MB3_D4_3_PU.pdf and is for convenience included below.



**Marine
Microbial Biodiversity,
Bioinformatics & Biotechnology**

Ocean Sampling Day Handbook

Version 1.0 of 27th June 2013

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List of abbreviations

ABS	Access Benefits Sharing
ANL	Argonne National Laboratory, USA
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
DTA	Data Transfer Agreement
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	Mariene Informatie Service, The Netherlands
MAT	Mutually Agreed Terms
Micro B3	Biodiversity, Bioinformatics, Biotechnology
Micro B3 IS	Micro B3 Information System
MTA	Material Transfer Agreement
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	Universitaet 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), <http://www.microb3.eu/>, is a highly interdisciplinary project intending to develop a standardized 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 OSD Handbook is a best practice guide describing procedures and policies on the marine sample collection, logistics and bioinformatics developed by the Micro B3 primarily for marine research Stations and Cruises contributing to the main sampling event of the Micro B3 project, the Ocean Sampling Day 2014.

The Ocean Sampling Day, <http://www.microb3.eu/osd>, is a simultaneous sampling campaign of the world's oceans taking place on the summer solstice (21st June) in the year 2014.

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 standardized way and accompanied by a standardized 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 2014 is an excellent use case for development Micro B3 infrastructures that will support not only the Ocean Sampling Day 2014 but also other future marine sampling enterprises.

In addition to providing best practices on the collection of marine samples and data, this Handbook provides essential guidelines (1) to users on how to deposit acquired Micro B3/OSD metadata and data, and (2) to Sequencing Centres on how to deposit sequence and read data.

Disclaimer: This Ocean Sampling Day Handbook, version 1.0, is a best practice guide at the time of release of the Handbook.

However, the Micro B3 consortium will continue to develop the Micro B3 Information System as well as integration level and data submission/retrieval systems of the involved data archives. In order to provide the marine sampling community with the latest information as the Micro B3 project evolves, this Ocean Sampling Day Handbook will have incremental versions.

Content of the Ocean Sampling Day Handbook

The Ocean Sampling Day Handbook intends to instruct users on the following topics described in individual chapters:

1. *Ocean Sampling Day (OSD) Workflow*
2. *OSD Site Registration*
3. *OSD Permits and Policy*
4. *OSD Sampling Instructions & Protocols*
5. *OSD Mandatory and Recommended Information*
6. *Sending Samples to Sequencing Centres*
7. *Bio-archiving Samples*
8. *Submitting Environmental Data and Sample Metadata to PANGAEA*
9. *Submitting Sample Metadata to ENA*
10. *Submitting Reads and Sequence Data to ENA*

References

Annex I OSD Logsheets

Annex II (a) MTA – Agreement between OSD Participant and ANL

(b) DTA – Agreement between ANL and EMBL-EBI

Annex III Draft Agreement between OSD Participant and SI NMNH

Annex IV Model Agreement on Access to Marine Microorganisms and Benefit Sharing

1. Ocean Sampling Day Workflow

A Sampling Site, i.e. marine research Station or Cruise, interested in participating in the Ocean Sampling Day 2014 shall follow the Micro B3 best practice workflow shown schematically in the **Figure 1** and described in the steps below:

1. Sampling Site will register for the OSD (please see the chapter *OSD Site Registration*)
2. Sampling Site will obtain required sampling permissions before the OSD sampling begins (please see the chapter *OSD Permits and Policy and the Annex II, III and IV*)
3. Sampling Site will download sampling OSD Logsheets (please see the *Annex I*)
4. **OSD sampling.**
Sampling Site will use for the OSD sampling standardized protocols and instructions (please see the chapter *OSD Sampling Instructions & Protocols and OSD Mandatory and Recommended Information*)
5. Sampling Site will dispatch samples to the Sequencing Centre (please see the chapter *Sending Samples to Sequencing Centres*).
The dispatched samples will be accompanied by:
a/ a copy of the OSD Logsheets
b/ SI NMNH shipping checklist for bio-archiving (please see the chapter *Bio-archiving Samples*)
6. Sampling Site will submit sample metadata (*mandatory Micro B3 checklist*) and environmental data to the Pangaia (please see the chapter *Submitting Environmental Data and Sample Metadata to PANGAEA*)
7. Sampling Site will submit sample metadata (*mandatory Micro B3 checklist*) to the ENA (please see the chapter *Submitting Sample Metadata to ENA*)
8. Sequencing Centre will submit reads and sequences to the ENA (please see the chapter *Submitting Reads and Sequence Data to ENA*). These molecular data will be linked to the submitted sample metadata at the ENA.

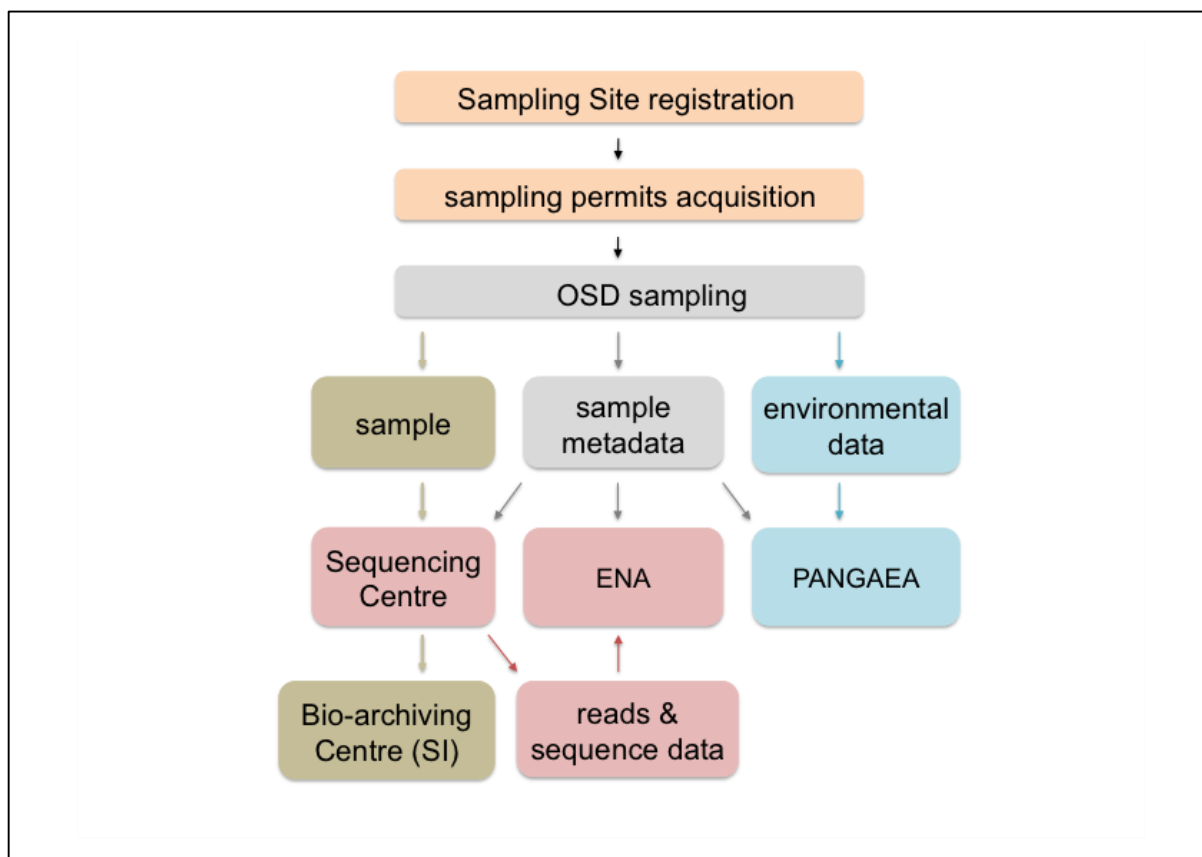


Figure 1: The Micro B3 best practice workflow for OSD Sampling Sites.

Sampling Stations and Cruises will need to register for the OSD and obtain required sampling permissions before the OSD sampling begins. Sampling Sites shall for the OSD samples use Micro B3-standardised protocols and sampling instructions. OSD samples will be cryo-preserved at the SI NMNH and also used for nucleotide sequence analysis. Sample metadata and environmental data will be archived at the PANGAEA. Sample metadata will also be archived at the ENA and linked to reads and sequence data provided by Sequencing Centre(s).

Micro B3 will integrate all environmental, morphological and molecular data obtained with/from biological samples collected during the Micro B3 OSD.

Metadata recorded by sampling groups during the OSD will be shared among environmental, biodiversity and sequence/read data archives.

The flow of data and samples is depicted in **Figure 2**. All environmental and morphological data together with their metadata (MANDATORY MICRO B3 CHECKLIST, see Chapter 5) will be archived at the PANGAEA (Data Publisher for Earth and Environmental Science; <http://www.pangaea.de>). Condensed summary of each OSD sampling event or cruise 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.marbef.org/data/eurobis.php>). All molecular data generated from Micro B3 samples together with their metadata (MANDATORY MICRO B3 CHECKLIST) will be archived at the ENA (European Nucleotide Archive at the European Molecular Biology Laboratory, European Bioinformatics Institute, <http://www.ebi.ac.uk/ena/>). These three information systems (SeaDataNet, ENA and

EurOBIS) will be connected to the integrated Micro B3 Information System, which will provide a primary access to all OSD data.

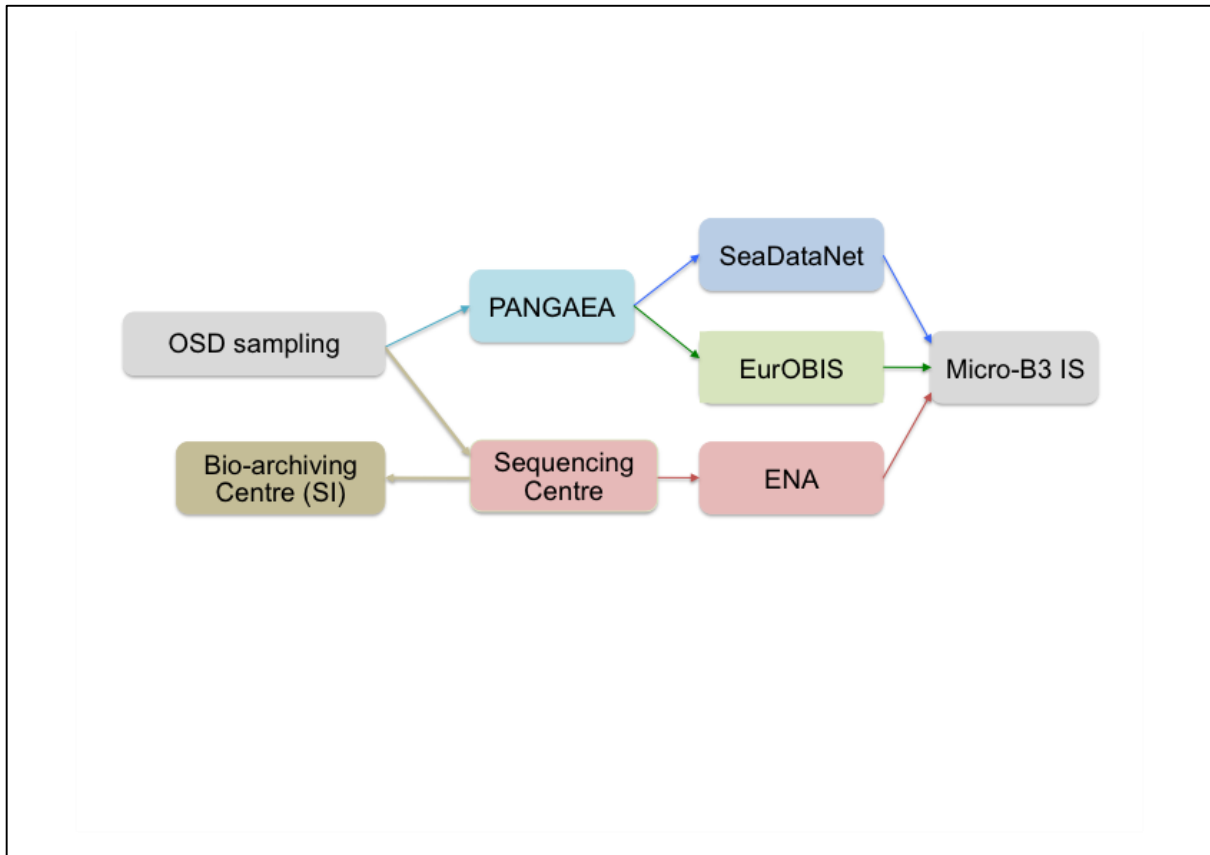


Figure 2: The Micro B3 OSD flow of samples and data.

The flow of samples from OSD Sampling Sites to Sequencing Centres and then further on to the SI NMNH bio-archive – **brown line**; the flow of environmental data from OSD Sampling Sites to the PANGAEA – **turquoise line**; the flow of oceanographic data from the PANGAEA to the SeaDataNet – **blue line** and biodiversity data to the EurOBIS – **green line**; the flow of reads and sequence data from Sequencing Centres to the ENA – **red line**.



2. OSD Site Registration

The Micro B3 project is leading an open call for participation in the Ocean Sampling Day 2014 (OSD). Scientists of marine research Cruises and Stations or non-scientists can get involved in the OSD. Sites that can provide minimal required information will be included into the OSD on the voluntary basis. An increasing number of Genomic Observatories form part of this growing OSD Sampling Sites network.

You can express your interest in joining the OSD 2014 at <http://oceansamplingday.blogspot.co.uk/2012/11/call-for-participation-in-ocean.html>

The OSD team is building a Micro B3 Catalogue of marine sampling Sites involved in the OSD with an overview of their sampling opportunities and expertise. Development of the catalogue will lead to the *OSD Sites Registry* where uniform description of the OSD Sites will be integrated with environmental and sequence data following the 2014 OSD event and will be maintained and publicly available via the Micro B3 IS.

Sites in the *OSD Sites Registry* will be formally considered part of the OSD consortium, will obtain registration accounts at the primary archives for OSD data submissions, i.e. PANGAEA and ENA, and will receive barcodes for the purpose of bio-archiving their OSD samples at the SI NMNH.

More information on the *OSD Sites Registry* can be found from the OSD website at <http://oceansamplingday.blogspot.co.uk/>

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

3. OSD Permits and Policy

3.1 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.

Based on the Micro B3 data policy, the OSD data sharing policy draws on needs of Micro B3 and OSD community, on existing policies (Biosharing, <http://biosharing.org/policies>) and on best practice according to the *12 Step Path do 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 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/welcomereport0303.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 working group.
- Publication: It is expected that authorship of the global analysis of the OSD dataset to belong to the OSD consortium. For specialist publication authorship will be defined on case-by-case basis.

Full description of the Micro B3 data policy is beyond a scope of this Handbook. However, groups involved in the Micro B3 principal sampling campaign, the OSD, or everyone submitting or using data of the Micro B3 consortium should be aware of the OSD data sharing policy, which is available from the OSD website, <http://oceansamplingday.blogspot.co.uk/>.



3.2 OSD Sampling Permits

The Micro B3 project provides a legal framework for the Ocean Sampling Day.

The marine scientific research activities need to be organized according to a set of protocols in order to respect national and international legal commitments in the States involved, and to achieve legal certainty that is beneficial for the research community, the provider countries and also the possible private investors.

Micro B3 has developed **Model Agreements 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. These Micro B3 model ABS agreements are in line with the Nagoya Protocol, not yet in force, and the Convention on Biological Diversity (CBD) that entered into force on 29 December 1993 and 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

The Micro B3 ABS model agreements are designed to cover the following different options: public domain utilization of the accessed genetic resources, full proprietary utilization and hybrid utilization of the accessed genetic resources.

A) PUBLIC DOMAIN CONTRACT: You envision 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.). The application of this clause implies that the knowledge resulting from research and development on the collected materials has to be publicly available. No patent rights can be granted and there should be no expenses to accede to this knowledge (apart from normal costs for dissemination). Every scientist with adequate expertise will be able to access the scientific knowledge/information resulting from the project.

If you wish in later stage of the research process, you can change the intent of the research and envision commercial uses. Such commercial uses are permitted, but the conditions of this should be negotiated later (consent clause under article 4.4). This clause is activated when the produced knowledge is used with exclusive protection, including products and processes developed.

(B) HYBRID: You envision public domain uses of some genetic resources / some use of genetic resources and you know some potential commercial uses for other genetic resources/other uses of the accessed genetic resource.

(C) PURE COMMERCIAL: You envision commercial use for all the genetic resources accessed and you decide to negotiate the benefit sharing conditions for commercial use upon the access of the genetic resources. In this case only use article 4.3. (and delete articles 4.2 and 4.4.).

By signing the agreement the provider country (1) gives the research consortium permission to sample in its waters and (2) enters into a partnership resulting in following automatic benefits for the provider country:

- access to scientific results through open-access integrated databases
- monetary benefits in case of proprietary use
- benefits from legal certainty provided by the agreement
- affiliation to a major international scientific bioinformatics network

Additional benefits can be negotiated with the provider country relating to sampling, data analysis and management:

- mentoring of the provider country scientists by Micro B3 scientists
- participation of the provider country scientists in research activities related to the sampling activity and its analysis
- participation of the provider country scientists in training for capacity building on bioinformatics, data management and data analysis
- sample archiving for defined period of time
- support for fund rising for sequencing, for finding sequencing partners and long-term archive partners

The Micro B3 ABS model agreements also contain a **viral license clause** for improved monitoring of material transfer and data transfer to third parties. The viral licence concept means that the ABS contract travels with the resource and the data upon transfer.

The Micro B3 model ABS agreements offer a viral license clause in article 5. This clause grants that all the obligations of the initial agreement will be imposed on subsequent use of the materials and the produced data when transferred. When the viral licence clause is used, then the scientist/scientific institution is allowed to transfer the material to third parties if they sign a new contract in which they commit themselves to respect the conditions of the initial ABS agreement. Every transfer to new third parties would require the signature of an MTA that makes the initial ABS agreement binding, to which the initial ABS should be attached as Annex. At each transfer however, according to the Nagoya Protocol, consent is required from the competent national authority in the provider country (Prior Informed Consent). Here two situations can be distinguished:

- If you use the viral licence 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 through negotiation.

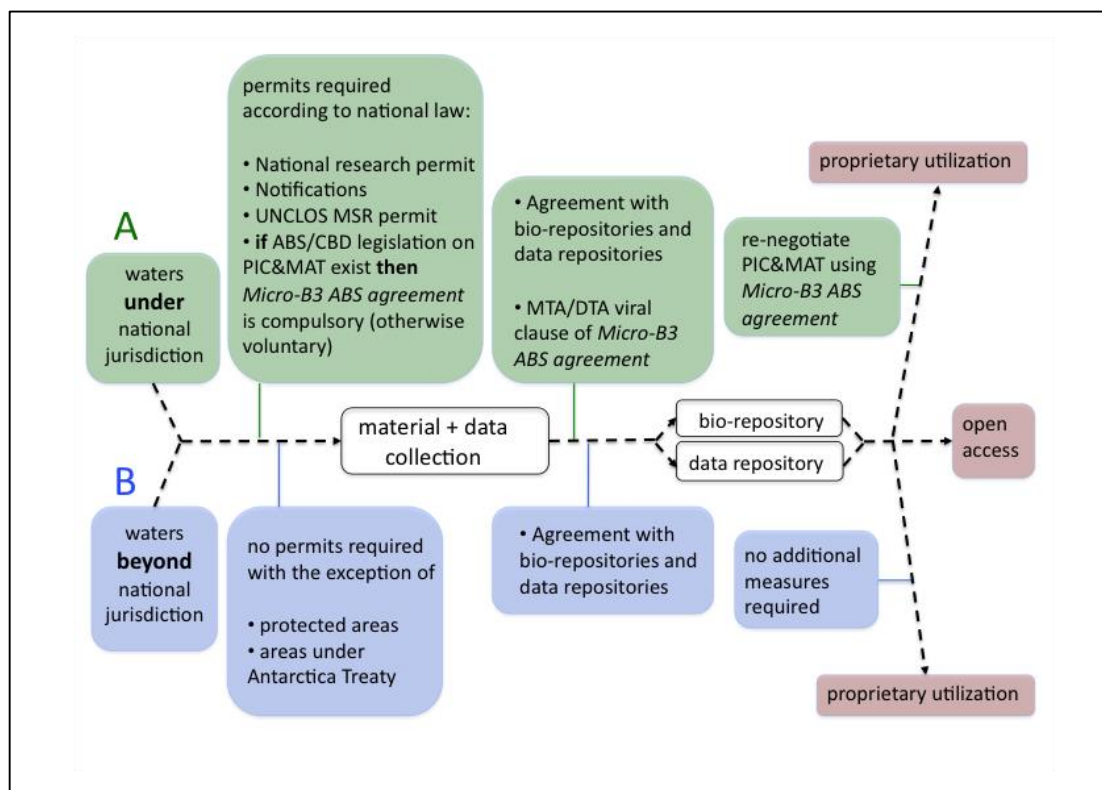


Figure 2. Overview of legal documents needed prior to the OSD as suggested by the Micro B3 consortium. Sampling in waters under national jurisdiction (**case A**); sampling in water beyond national jurisdiction (**case B**).

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

The Figure 2 summarizes a **legal workflow for the OSD participants** regarding the legal steps to be undertaken in accessing the genetic resources and in transferring material and data to third parties, bioarchiving institution or sequencing institutions. The three options covered by the three different MICRO B3 model ABS agreements are illustrated.

For access, two cases can be distinguished depending on whether the sampling event occurs in waters under national jurisdiction (case A) or in areas beyond national jurisdiction (case B). In the case A different documents might be needed according to the national legislation of each particular country where the sampling takes place: these may include¹ research permit; wildlife permit; other permit; simple notification; an ABS agreement negotiated on the basis of the ABS/CBD legislation on Prior Informed Consent and Mutually Agreed Terms if this legislation is in place. If this is the case then the OSD Institution willing to sample there will have to undertake negotiation with the competent authority on the basis of the Micro B3 ABS agreement. The model agreement might be accepted as it is (in one of the three options) and signed by the competent authority. It might be the case that the country has its own model agreement or proposes one: in this case the main features of MICRO B3 ABS model agreement

¹ It might be the case that different permits are required from different authorities therefore it is very important to be aware of the national legislations in place in the country and of the authorities that are competent to issue such permits or to negotiate agreement.



(public domain condition – viral license clause and renegotiation clause in case of change of intent) would be compulsory and they should be reflected in the ABS agreement negotiated with the country. There might also be the case where the country does not accept one, or more, of these clauses, and this might lead you to opt for not sampling in that country.

Once the permits are issued and/or the ABS agreement is signed the material can be accessed in accordance with the permits and/or the agreement.

Once the material is collected and sampling-related data recorded the MTA and DTA containing the viral clauses of the Micro B3 model ABS agreements will be needed in the case A of sampling in waters under national jurisdiction (see **Annex II**).

Moreover, both in case A and in case B another MTA agreement with the bio-repository institution accepting the collected material and another DTA with the data archive accepting the collected data might be needed: in fact 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 (See **Annex III** – MTA with the Smithsonian Institution).

Procedure for access to genetic resources

1. Well in advance, at least 12 months ahead, contact the National Focal Point for the Convention on Biological Diversity (CBD) in the country where you are willing to sample. Contact the Embassy of the foreign country if you are willing to sample in foreign waters. Ask for an advice on documents needed according to national law of the country.

2. If the country has ABS/CBD legislation requiring PIC and MAT contact the competent national authority and start negotiating an ABS agreement on the basis of Micro B3 ABS model agreement.

More information on national ABS measures can be found at <http://www.cbd.int/abs/measures/>.

3. If there are other notifications or permits needed make sure they are in place prior to the OSD event.

In summary, the legal steps are the following:

- Original agreement to access the sample (full template in Annex IV) and any other permit/authorization/notification required according to the national legislation
- Agreement to transfer the sample (Material Transfer Agreements), i.e. clause 5.1 of the template and a copy of the original agreement in attachment
- Agreement to transfer data (Data Transfer Agreement), i.e. clause 5.2 of the template and a copy of the original agreement in attachment

4. OSD Sampling Instructions & Protocols

4.1. Environmental Parameters

Micro B3/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, and to support hypothesis-driven analyses.

Since OSD is a global sampling campaign where scientific interests of contributing Sites cover a broad spectrum, Micro B3 has developed a **Micro B3 list of OSD mandatory and recommended ENVIRONMENTAL PARAMETERS, Table 1**, describing the marine environmental conditions of an OSD sample.

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 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 nine 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.

Micro B3 consortium 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 mandatory (highlighted) and recommended ENVIRONMENTAL PARAMETERS describing the marine environment of a Sample. Recommendation applies to OSD marine research Cruises and Stations sampling in the pelagic zone.

ENVIRONMENTAL PARAMETERS	CATEGORY	PARAMETER	DESCRIPTION	Control vocabulary/format *
	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 Optical Properties	Downward PAR	Visible waveband radiance and irradiance measurements in the water column	SDN:P02:75:VSRW SDN:P06:46:UMES for µE/m ² /s	
	Turbidity	Transmittance and attenuation 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:UGPL 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/Microp plankton	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/Macrop plankton	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
		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>)



4.2. Samples for Genomics

Micro B3/OSD participants are encouraged to sample plankton from three size-fractions, corresponding to three taxonomic groups:

- Unicellular Eukaryotes (3 μm – 200 μm)
- Unicellular Prokaryotes (0.22 μm – 3 μm) or (>0.22 μm)
- Viruses (<0.22 μm)

Because Micro B3/OSD participants have various means and capacity to sample for genomics we propose three protocols that can be combined “à la carte”. These three protocols are shown on **Figure 3** and consist of (A) Collecting Prokaryotes on 0.22 μm filters using Sterivex cartridges; (B) Collecting unicellular Eukaryotes on 3 μm membranes and Prokaryotes on 0.22 μm membranes; and (C) Collecting Viruses from the <0.22 μm filtrate. We recommend doing (A) + (B) + (C) as much as possible, but these protocols can be combined in various ways:

- ✓ (A) or (B)
- ✓ 2x (A) or 2x (B)
- ✓ (A) + (B)
- ✓ (B) + (C)
- ✓ 2x (B+C)
- ✓ (A) + (B) + (C)
- ✓ Etc....

Sampling instructions and protocols were agreed upon by the Micro B3 consortium and correspond to standard sampling protocols used at the L4 station (Protocol A) and during the Tara-Oceans expedition (Protocols B & C). Micro B3/OSD participants are welcome to take additional samples using their local methods.

Please record any modification to the protocols on the OSD Logsheets described in the Annex I of this handbook and available online upon registration to the OSD.

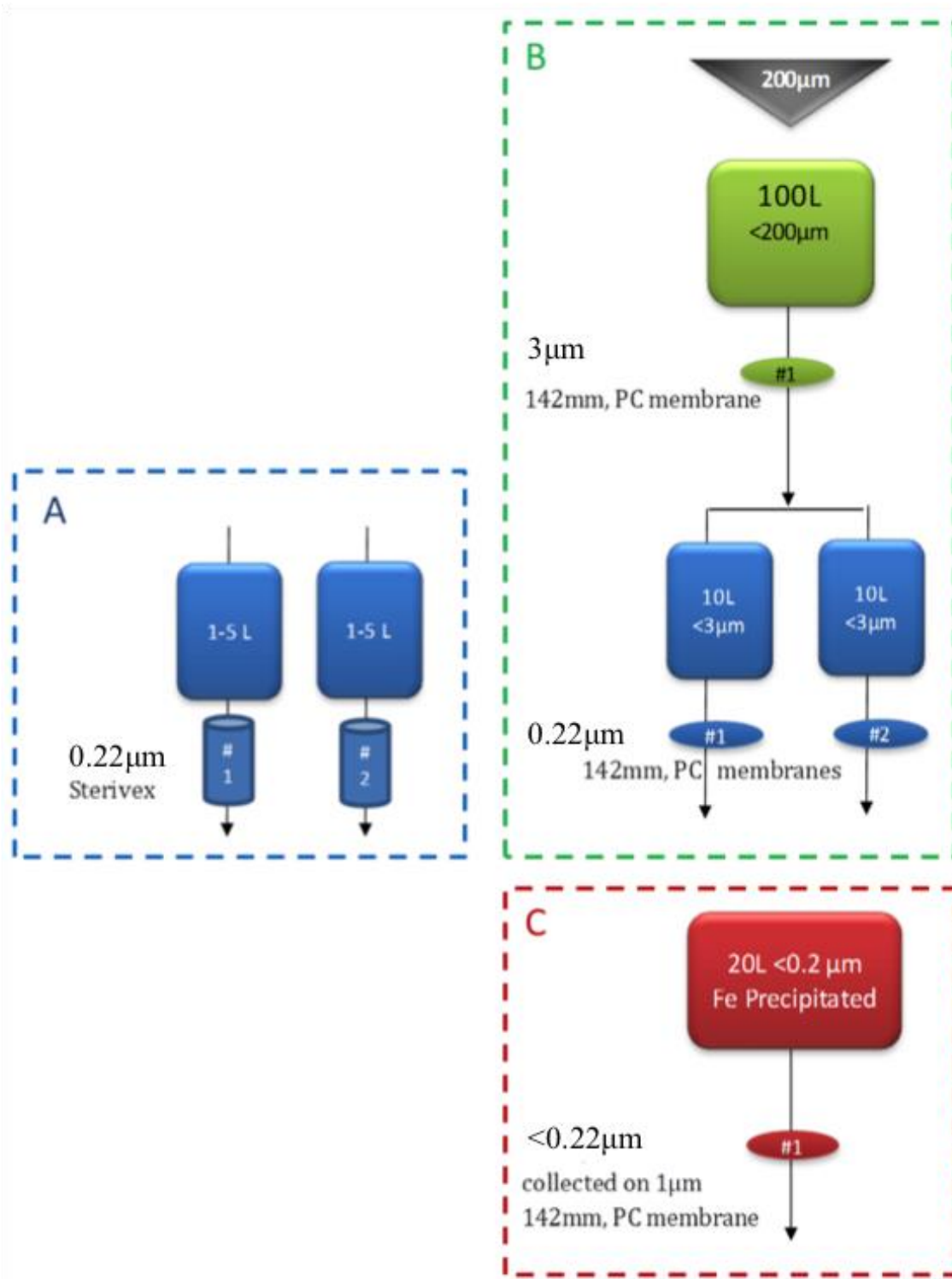


Figure 3: Three Micro B3/OSD protocols that can be combined “à la carte”; (A) Collecting Prokaryotes on 0.22 µm filters using Sterivex cartridges; (B) Collecting unicellular Eukaryotes on 3 µm membranes and Prokaryotes on 0.22 membranes; and (C) Collecting Viruses from the <0.22 filtrate.

PROTOCOL (A) Collecting Prokaryotes on 0.22 µm pore size filters using Sterivex cartridges

Sampling

Isolate 10-20L of seawater using a Niskin bottle or 10% acid washed bucket from the surface (0-2 m depth) of the water column. Collect a minimum of 4 replicate samples for which as minimum the MANDATORY MICRO B3 CHECKLIST, Table 2a, is known. If possible, for each time zone take the samples between 10am and 2pm.

OSD PROTOCOL (Source: Western Channel Observatory)
for **NUCLEOTIDES** analysis of unicellular **PROKARYOTES**
in particulate matter, size-fraction >0.22µm
from sea **WATER**

Reference: Gilbert *et al.* (2010);

SAMPLE_Protocol_ID: NUC_PROK_W0.22>

SAMPLE_Protocol_Short_Label (to write on barcode stickers): NPL022

SAMPLE_Size-Fraction_Upper-Threshold: >>>

SAMPLE_Size-Fraction_Lower-Threshold: 0.22 µm; Keep filtrate for NUC_VIR_W<0.22 as needed

SAMPLE_Material: Particulate matter on a 0.22 µm pore size filter

SAMPLE_Container: Sterivex cartridge
(<http://www.millipore.com/catalogue/item/svgv010rs>)

SAMPLE_Treatment_Chemicals: none

SAMPLE_Treatment_Storage: Liquid Nitrogen

SAMPLE_Filtration_Volume: 10-20 L

SAMPLE_Filtration_Time: 15 min

Procedure for each replicate:

- ✓ Filter 1-5L of seawater to collect particulate matter on a 0.22 µm pore size filter, using Sterivex cartridges (see URL above for purchase reference); Instructions on how to use Sterivex cartridges can be viewed at the video of the MIRADA project at <http://amarallab.mbl.edu/mirada/mirada.html>;
- ✓ Filtration for Sterivex should be done using either a peristaltic pump or a hand pump (e.g. 50 mL sterile syringe). In either case, use a “luer lock” adapter to attach the Sterivex;
- ✓ The Sterivex should be pumped free of standing water following filtration but does not need to be dry;



- ✓ Seal both ends of the Sterivex cartridge using “sticky tack” (“blue tack”, “Gommette”). Parafilm will crumble at -80°C and therefore should not be used;
- ✓ Label the sample, place in a sterile plastic bag and freeze 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.

Please record on the logsheets:

- ✓ Volume of seawater that you filtered
- ✓ Time taken to filter the sample
- ✓ Observations about the colour of the filter

PROTOCOL (B) Collecting unicellular Eukaryotes on 3 µm pore size filters and Prokaryotes on 0.22 µm pore size filters

General Recommendations Regarding Large Volume Filtrations

Before starting sampling flush the filtration system and water containers (carboys) with diluted bleach and then freshwater. Clean surfaces, tweezers and everything you use with ethanol before starting filtration. Always wear gloves (unlike the pictures below!). Handle filters carefully, pick them by the edge and do not touch the part of the filter that will be in touch with the sample. Make sure that the filters are centrally positioned in the filter holders.

Pumping is done preferably using a peristaltic pump and 142 mm filter holders, which can be set up in series to optimise sample processing times. The tubing that goes in the pump head **MUST** be Masterflex silicon tubing. The inlet & outlet tubes must be securely fixed. It may be necessary to put adjustable metal collars.

Gently turn on the pump until some water is in contact with the filter, then turn up the flow rate. It may be necessary to remove trapped air by raising the bleed valve lever before a high flow rate can be maintained. When the filtration is finished, turn off the pump and release the pressure (bleed valve) before opening filter holders. Once the filter holder is open, water remaining on top of the filter may be removed using a manual vacuum pump connected to the outflow. Membranes are folded in half then folded again.



Preparation: Rinse 1x100L carboy (Eukaryotes) twice with sample seawater before filtration. Prepare the filtration system to be ready to start filtering as soon as water sample is collected. Use the peristaltic pump with a pressure gauge (maximum pressure = 11-13 psi). Rinse the two 10L carboys (Prokaryotes) twice with the filtrate of the Eukaryotes filtration (i.e. <3 µm). Fill the 100-L carboy with seawater prefiltered on 200 µm, using a sieve or a funnel lined with a 200 µm nylon mesh.

In line serial filtration: <200-µm Seawater can be filtered in series through two 142mm filtration tripods; the first one holding a 142mm PC 3µm membrane; and the second one holding a 142mm Millipore Express Plus 0.22µm filter. Collect the filtrate in two 10-L carboys or a graduated 20-L carboy in order to know the volume of water that passed through the 0.22 µm membrane. Keep 20 L of filtrate for the Protocol (C), if needed, and discard the rest of the filtrate. Upon starting the pump, purge air from system and filter water during 15min. Make sure no air enters the system, which could rupture filters. If ruptured, stop pumping, purge air and replace with a new one. If flow rate decreases considerably during filtration, first check all the connections. If this does not improve the filtration, the filters are possibly clogged. Usually, with the Express Plus filter we can filter about 100L in an hour. Change the first filter and if the flow rate is still low change the second one in line.



Two-steps serial filtration: <200- μm Seawater is filtered first on a 142mm filtration tripod holding a 142mm PC 3 μm membrane, and the filtrate is collected in two 10-L carboy for the Prokaryotes filtration. Upon starting the pump, purge air from system and filter water during ca. 60 min at a rate of ca. 1L/minute. The filtrate is then passed on a 142mm filtration tripod holding a 142mm PC 0.22 μm membrane, and the filtrate is collected in two 10-L carboy for the Prokaryotes filtration. Upon starting the pump, purge air from system and filter water during 15min at a rate of ca. 1L/minute. Make sure no air enters the system, which could rupture filters. If ruptured, stop pumping, purge air and replace with a new one. After filtration, flush the system with freshwater or sterile MilliQ water.

OSD PROTOCOL (Source: Tara-Oceans consortium)
for **NUCLEOTIDES** analysis of unicellular **EUKARYOTES**
in particulate matter, size-fraction **3-200 μm**
from sea **WATER**

Reference: Not *et al.* (in preparation)

SAMPLE_Protocol_ID: NUC_EUK_W3-200

SAMPLE_Protocol_Short_Label (to write on barcode stickers): NE3200

SAMPLE_Size-Fraction_Upper-Threshold: 200 μm ; Use a sieve or a funnel lined with a 200 μm nylon mesh

SAMPLE_Size-Fraction_Lower-Threshold: 3 μm ; Use the filtrate for NUC_PROK_W0.22-3

SAMPLE_Material: Particulate matter on a 142mm, 3 μm pore size, polycarbonate membrane (TSTP14250)

SAMPLE_Container: 5-mL cryovial

SAMPLE_Treatment_Chemicals: none

SAMPLE_Treatment_Storage: Liquid Nitrogen

SAMPLE_Filtration_Volume: 100 L

SAMPLE_Filtration_Time: 15 min

Procedure for each replicate:

- ✓ Remove the PC 3 μm filter and place it carefully in a 5-mL cryovial;
- ✓ Store immediately in Liquid Nitrogen;
- ✓ NOTE ON LOGSHEETS the filtration times and volumes.



OSD PROTOCOL (Source: Tara-Oceans consortium)
for **NUCLEOTIDES** analysis of unicellular **PROKARYOTES**
in particulate matter, size-fraction **0.22-3µm**
from sea **WATER**

Reference: Not *et al.* (in preparation)

SAMPLE_Protocol_ID: NUC_PROK_W0.22-3

SAMPLE_Protocol_Short_Label (to write on barcode stickers): NP0223

SAMPLE_Size-Fraction_Upper-Threshold: 3 µm; Use filtrate from NUC_EUK_W3-200

SAMPLE_Size-Fraction_Lower-Threshold: 0.22 µm; Keep filtrate for NUC_VIR_W<0.22 as needed

SAMPLE_Material: Particulate matter on a 142mm, 0.22µm, Millipore Express Plus membrane (GPWP14250)

SAMPLE_Container: 5-mL cryovial

SAMPLE_Treatment_Chemicals: none

SAMPLE_Treatment_Storage: Liquid Nitrogen

SAMPLE_Filtration_Volume: 10 L

SAMPLE_Filtration_Time: 15 min

Procedure for each replicate:

- ✓ Remove the PC 0.22 µm membrane and place it carefully in a 5-mL cryovial;
- ✓ Store immediately in Liquid Nitrogen;
- ✓ NOTE ON LOGSHEETS the filtration times and volumes.

PROTOCOL (C) Collecting Viruses from <math><0.22\ \mu\text{m}</math> filtrate

General Recommendations Regarding Large Volume Filtrations

Before starting sampling flush the filtration system and water containers (carboys) with diluted bleach and then freshwater. Clean surfaces, tweezers and everything you use with ethanol before starting filtration. Always wear gloves (unlike the pictures below!). Handle filters carefully, pick them by the edge and do not touch the part of the filter that will be in touch with the sample. Make sure that the filters are centrally positioned in the filter holders.

Pumping is done preferably using a peristaltic pump and 142 mm filter holders, which can be set up in series to optimise sample processing times. The tubing that goes in the pump head **MUST** be Masterflex silicon tubing. The inlet & outlet tubes must be securely fixed. It may be necessary to put adjustable metal collars.

Gently turn on the pump until some water is in contact with the filter, then turn up the flow rate. It may be necessary to remove trapped air by raising the bleed valve lever before a high flow rate can be maintained. When the filtration is finished, turn off the pump and release the pressure (bleed valve) before opening filter holders. Once the filter holder is open, water remaining on top of the filter may be removed using a manual vacuum pump connected to the outflow. Membranes are folded in half then folded again.



Preparation: Treat the 20L of $0.22\ \mu\text{m}$ filtrate with FeCl_3 to precipitate the viruses: Add 2mL of 10g Fe/L stock solution to 20 L of filtrate (final concentration of 1mg Fe in the seawater). Shake vigorously for 1 min after addition of FeCl_3 and repeat shaking several times. Let sit for 1 hr at RT (can sit longer at $+4^\circ\text{C}$ if needed, up to overnight).

Filtration: Clean the filter holder by running 500ml of MiliQ water, followed by 500ml of FeCl_3 -treated filtrate through it (without the filters). Then, with the cleaned filter rig, filter the FeCl_3 -treated viral fraction sample onto a $1.0\ \mu\text{m}$, 142mm, polycarbonate (PC) membrane filter on top of a $0.8\ \mu\text{m}$, 142mm, Supor filter (polyethersulfone available from Pall Life Sciences, cat. no. 60114.). Use the peristaltic pump with a pressure gauge to keep the maximum pressure $< 15\ \text{psi}$; 10-15psi can force leakage with the Polycarbonate 142mm filter.

The FeCl_3 precipitate (with viruses) will be captured on the PC filter. The Supor filter is just for support in the 142mm stainless steel filtration apparatus, so it is discarded after filtration. To filter 20L of FeCl_3 -treated seawater you may need up to 3x 142mm $1.0\ \mu\text{m}$ PC membrane (deep samples usually need only 1, whereas surface samples usually need 3); when the filter starts to clog and the pressure goes above 15 psi, you can stop the peristaltic pump and change the PC membrane before resuming filtration. It is usually not necessary to use more than one Supor membrane for a sample since it is only for support and does not get clogged.

Post-processing: Resuspend the viral precipitate by adding 10mL of a Resuspension buffer to the filter shaking occasionally to distribute the buffer over the filters. The viral concentrate can be used for isolation of intact viruses or viral DNA/RNA. **Resuspension buffer:** 17 mL MQ-water; 6.25 mL 1M Tris base; 5 mL 1M EDTA (use disodium, dihydrate EDTA); 10 mL 1M MgCl₂; 10 mL 1M Ascorbic acid; adjust to pH=6 to 6.5 with ca. 2 mL 5M NaOH (Note: It is best to prepare buffer just prior to use, but store at 4°C and use within 2 days. A precipitate may form before the pH adjustment, but final buffer should be clear without a precipitate.)

OSD PROTOCOL (Source: Tara-Oceans consortium)
for **NUCLEOTIDES** analysis of **VIRUSES**
in precipitated particulate matter, size-fraction **≤0.22 μm**
from sea **WATER**

Reference: Not *et al.* (in preparation)

Reference : Seth *et al.* (2011)

SAMPLE_Protocol_ID: NUC_VIR_W<0.22

SAMPLE_Protocol_Short_Label (to write on barcode stickers): NPU022

SAMPLE_Size-Fraction_Upper-Threshold: 0.22 μm; Use filtrate from NUC_PROK_W0.22-3

SAMPLE_Size-Fraction_Lower-Threshold: <<<

SAMPLE_Material: Particulate matter on a 142mm, 1 μm, polycarbonate membrane (GPWP14250)

SAMPLE_Container: 5-mL cryovial

SAMPLE_Treatment_Chemicals: none

SAMPLE_Treatment_Storage: Liquid Nitrogen

SAMPLE_Filtration_Volume: 10 L

SAMPLE_Filtration_Time: 60 min

Procedure for each replicate:

- ✓ Remove the PC 1 μm membrane, fold it and place it carefully in a 5-mL cryovial;
- ✓ Store immediately in Liquid Nitrogen;
- ✓ NOTE ON LOGSHEETS the filtration times and volumes.

5. OSD Mandatory and Recommended Information

5.1 Micro B3/OSD Mandatory Information



One Micro B3/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 Micro B3/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 Micro B3 dataset has to therefore contain a minimal core of classifiers allowing relating one otherwise disparate data type to another. This minimal sample metadata that will further be referred to as the **MANDATORY MICRO B3 CHECKLIST** will ensure that each OSD sample is accompanied by minimal essential contextual information and that Micro B3 data can be integrated among independent data resources.

The **MANDATORY MICRO B3 CHECKLIST, Table 2a**, is the minimal reporting standard accompanying every Micro B3/OSD-collected sample.

The **MANDATORY MICRO B3 CHECKLIST** must be submitted to both, the ENA and the PANGAEA.

Table 2a: Micro B3 checklist of OSD mandatory 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;	OSD-SS2014
	SAMPLING_Site	Refers to the site/station where data/sample collection is performed.	Terms list: OSD Sites Registry**	Crete Time Series Station
	SAMPLING_Platform	Refers to the large infrastructure from which data/sample collection is performed, e.g. a ship or a coastal observatory.	Free text;	Poseidon-E1-M3A
	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	035.6666E 035.6702E
	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	24.6666N 24.6643N
	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	14.7 m
	SAMPLE_Protocol_Label	Identifies the protocol used to produce the sample, e.g. filtration and preservation.	Term list; See the SAMPLE_Protocol_Short_Label in the OSD Protocols Section for details.	NP0223

SAMPLE_ ID	A unique identifier (barcode) for each sample, e.g. one ID for each filter generated during sampling. The IDs are generated, printed and sent to the sampling groups following their registration to OSD. See the Registration Section for details.		SI NMNH barcode ID
ENVIRONMENT_ Biome	Refers to classes of ecologically similar communities of plants, animals, and other organisms.	Terms list: EnvO (v1.53)	ENVO:00000447 for “marine biome”
ENVIRONMENT_ Feature	Refers for example to geographic features, or natural & artificial habitats that characterise the environment.	Terms list: EnvO (v1.53)	ENVO:00000569 for “marine habitat”
ENVIRONMENT_ Material	Refers to the matter that was taken from the environment by the sampling event.	Terms list: EnvO (v1.53)	ENVO:00002042 for “surface 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: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>)

** OSD Sites Registry can be accessed from the OSD website. (<http://oceansamplingday.blogspot.co.uk/>)

5.2 Micro B3/OSD Recommended Information

The MANDATORY MICRO B3 CHECKLIST is the minimal sample metadata associated with each OSD sample. However, the MICRO B3 CHECKLIST lies on a multi-disciplinary crossroad of research domains and on its own is not fully compliant with standards of the three domains (oceanographic, genomic and biodiversity). **Figure 4** shows schematically the MANDATORY MICRO B3 CHECKLIST surrounded by a sphere of mandatory, recommended and optional information elements in standards of each domain.

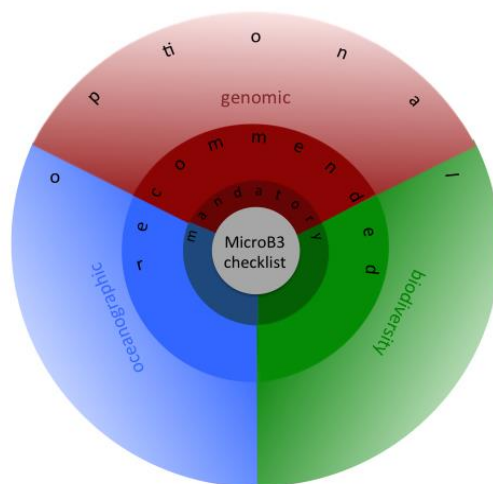


Figure 4: Merging standards from multiple scientific domains under Micro B3.

Micro B3 has identified additional information that, when reported, will offer a number of assets to data providers. Reporting additional information, Tables 2b – 2g, will:

- a. Assure 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. Allow OSD samples to be sequenced and molecular data deposited in a sequence data archive, the ENA.
- c. Allow 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. Allow 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.

Micro B3 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)**, **MEASUREMENT (Table 2f)** and **ORGANISMS** of a sample (**Table 2g**).

Table 2b: Micro B3 checklist of OSD mandatory (highlighted) and recommended information about SAMPLING.

Recommendation applies to OSD marine research Cruises and Stations sampling in the pelagic zone.

Field	Description	Control vocabulary/format	Example
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;	OSD-SS2014
SAMPLING_Site	Refers to the site/station where data/sample collection is performed.	Terms list: OSD Sites Registry**	Crete Time Series Station
SAMPLING_Platform	Refers to the large infrastructure from which data/sample collection is performed, e.g. a ship or a coastal observatory.	Free text	Poseidon-E1-M3A
SAMPLING_Authors	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.	Format: <LASTNAME>, <FirstName>, <Institution>, <email>	JONES, Peter, Institute1, pjones@institute1.eu; SMITH, Mary, Institute2, msmith@institute2.eu
SAMPLING_Project	Refers to the project that organised/funded the data/sample collection.	Free text;	Micro B3
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

** OSD Sites Registry can be accessed from the OSD website. (<http://oceansamplingday.blogspot.co.uk/>)

Table 2c: Micro B3 checklist of OSD mandatory (highlighted) and recommended 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	035.6666E 035.6702E
	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	24.6666N 24.6643N
	EVENT_ID	A unique label describing the sampling event. It is automatically generated during data submission by the data brokering system.	Concatenation of four EVENT Fields: <CAMPAIGN>_<PLATFORM>_<DEVICE>_ <DATE/TIME>	OSD-SS2014_VLIZ- 330_Niskin_2014-06- 21T14:05:22Z
	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
	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: Micro B3 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
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	14.7 m
	SAMPLE_ Protocol_Label	Identifies the protocol used to produce the sample, e.g. filtration and preservation.	Term list; See the <i>SAMPLE_Protocol_Short_Label</i> in the OSD Protocols Section for details.	NP0223
	SAMPLE_ ID	A unique identifier (barcode) for each sample, e.g. one ID for each filter generated during sampling. The IDs are generated, printed and sent to the sampling groups following their registration to OSD. See the OSD Site Registration Section for details.		SI NMNH barcode ID
	SAMPLE_ Quantity	Refers to the quantity of environment that was sampled, most often with dimensions Length, Amount, Mass or Time.	Free text ; (=Sample size @ OBIS)	100 L
	SAMPLE_ Container	Refers to the container in which the sample is stored prior to analysis.	Term list; See the <i>SAMPLE_Container</i> in the OSD Protocols Section for details.	Cryovial, 5 mL
	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 <i>SAMPLE_Material</i> in the OSD Protocols Section for details. (=Investigation_type @ ENVO)	Particulate matter on a 142mm PC membrane
	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 <i>SAMPLE_Size-Fraction_Upper-Threshold</i> in the OSD Protocols Section for details.	3 µm

	SAMPLE_ Size-Fraction_ Lower-Threshold	Refers to the mesh/pore size used to retain the sample. Materials smaller than the size threshold are excluded from the sample.	Term list; See the <i>SAMPLE_Size-Fraction_Lower-Threshold</i> in the OSD Protocols Section for details.	0.22 μm
	SAMPLE_ Treatment_ Chemicals	Refers to the chemicals added to the sample, in the container, preservatives.	Terms list: ChEBI; See the <i>SAMPLE_Treatment_Chemicals</i> 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 <i>SAMPLE_Treatment_Storage</i> in the OSD Protocols Section for details.	Liquid nitrogen

Table 2e: Micro B3 checklist of OSD mandatory (highlighted) and recommended 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
INFORMATION ABOUT ENVIRONMENT OF A SAMPLE	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 http://www.marineregions.org/ (=Sea Region/Country @ EnvO)	Crete Sea
	ENVIRONMENT_Biome	Refers to classes of ecologically similar communities of plants, animals, and other organisms.	Terms list: EnvO (v1.53)	ENVO:00000447 for “marine biome”
	ENVIRONMENT_Feature	Refers for example to geographic features, or natural & artificial habitats that characterise the environment.	Terms list: EnvO (v1.53)	ENVO:00000569 for “marine habitat”
	ENVIRONMENT_Material	Refers to to the matter that was taken from the environment by the sampling event.	Terms list: EnvO (v1.53)	ENVO:00002042 for “surface 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_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		

*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>)

Table 2f: Micro B3 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.	URN:LSID Terms list: marinespecies.org	urn:lsid:marinespecies.org:taxname:345516
	ORGANISM_ Taxon_Scientific_Name	The full name of the lowest level taxon.	Obtained from urn:lsid:marinespecies.org:taxname:xxxxxx	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; Terms list: TBD	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.		

Table 2g: Micro B3 checklist of OSD mandatory (highlighted) and recommended 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:P011:353:xxxxxxx	SDN:P011:353: OSEDZZZZ for Concentration of suspended particulate material (organic) per unit volume of water
	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: marinespecies.org; 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

*SDN:P011:353 is a controlled Terms list describing fine-grained “WHAT” is measured. (<http://www.seadatanet.org/urnurl/SDN:P06:46:XXXX>)

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

6. Sending Samples to Sequencing Centres

OSD samples will be available for sequence analysis in order to accomplish one of the main Micro B3 goals, to create large-scale data on marine viral, bacterial and protistan genomes and metagenomes visualised in a rich environmental context.

Micro B3/OSD sampling Stations and Cruises shall send their OSD samples for nucleotide sequence analysis to the Sequencing Centre that offered to process the OSD-collected samples.

The Sequencing Centre has two tasks:

1. Generate reads/sequence data and send them to the ENA, where these molecular data will be linked to the Micro B3/OSD sample metadata information (MANDATORY MICRO B3 CHECKLIST) and made publically available to the Micro B3 Information System and downstream data analysis.
2. Pass OSD samples on to the SI NMNH for bio-archiving.

The Argonne National Laboratory, USA, offered to sequence prokaryotic OSD samples. The ANL will perform Illumina amplicon sequencing of bacterial community profiles for the hypervariable domains V4, V5 and V6 of the 16S rRNA gene, as described at

<http://www.nature.com/ismej/journal/vaop/ncurrent/full/ismej20128a.html>

The Argonne National Laboratory will send the samples on to the SI NMNH for bio-archiving.

A Material Transfer Agreement between each Micro B3/OSD participant and the ANL has to be signed prior to the OSD campaign and can be found in the **Annex II (a)** of this Handbook.

Instructions for shipping Micro B3/OSD samples to the Argonne National Laboratory:

1. Sterile-packed filters should be accompanied by a **copy of the OSD Logsheets**
2. Pack you filters on dry ice
3. Fill the declaration form that the package has no commercial value
4. Fill the custom clearance form, where the content should be 'water'
5. Complete the SI NMNH biorepository shipping checklist (see Chapter 7)
6. Email Sarah Owens Sarah.Owens@anl.gov as soon as you have dispatched your package
7. Ship the samples to
Sarah M. Owens, M.S.
Technical Director, IGSB-NGS Core
Argonne National Laboratory
9700 S. Cass Avenue
Bldg. 202, Rm. A353
Lemont, IL 60439
Phone number 630 252 2101



7. Bio-archiving 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.

Currently an agreement for the Micro B3/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 the next five years and make these collections available for research.

A Material Transfer Agreement between each Micro B3/OSD participant 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 barcodes for OSD samples that will each OSD Station or Cruise receive during registration process for the OSD 2014 event.

Micro B3/OSD sampling Stations or Cruises don't need to send samples to the SI NMNH directly.

Micro B3/OSD sampling Stations and Cruises shall complete the SI NMNH shipping checklist, described below and send it together with their OSD samples to the Sequencing Centre (see Chapter 6).

SI NMNH Biorepository Shipping Checklist for the Micro B3/OSD participants

1. The Micro B3 Ocean Sampling Day (OSD) sample is contained in a Sterivex, or equivalent dimension filter cartridge (p/n Millipore SVGV010RS), which has the ends blocked with sterile, inert material.
2. The Micro B3 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).
3. OSD Members understand that samples should not be sent directly to NMNH.
4. Argonne Labs should give a **minimum** of 72 hours advanced notice **prior** to shipment (please e-mail NMNHBiorepository@si.edu to arrange a good date for shipping—avoiding holidays, vacations, etc.).
5. All necessary permits (collecting, import/export) are included with the shipment (this provides proof that all samples were collected legally).
6. 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>).
7. Metadata on the sample is provided electronically in a tab or comma-delimited file. Expected data fields include: collector name, collector institution, collector or other sample identifying number, sample's country of origin, collection method, date collected (day-month-year), specific locality (using Universal Transverse Mercator or UTM code), elevation/depth, and any restrictions.

8. Submitting Environmental Data and Sample Metadata to PANGAEA

OSD sampling Stations and Cruises should submit the MANDATORY MICRO B3 CHECKLIST and their environmental and morphology-based biodiversity datasets to the PANGAEA (<http://www.pangaea.de/submit/>).

Registration to the PANGAEA's client system is required and may be in the future streamlined with the OSD registration process.

The online data submission form of the PANGAEA is shown below. The only mandatory fields in the form are "Authors" and the label "Micro B3/OSD".

All information in the **OSD Logsheets, Annex I**, should be digitized. One to many data files must be attached to the submission. The information required by the MANDATORY MICRO B3 CHECKLIST (Table 2a) can be included in the attached files or pasted in the "Description" field of the online form.



PANGAEA®
Data Publisher for Earth & Environmental Science

Logged in as **pesant** ([log out](#), [profile](#))

Submit Data to PANGAEA

Welcome to the PANGAEA data submission system. Any data from earth and life sciences are accepted. We highly appreciate you archiving and publishing your data with PANGAEA.



Benefits. Published data are fully citable and can be cross-referenced with journal articles – [hide more...](#)

- Long term preservation of data. PANGAEA is member of the [World Data System \(WDS\)](#) of the [International Council for Science \(ICSU\)](#).
- Quality assurance on metadata, such as citation, references, geo-location, and standard parameter vocabularies.
- Reliable identification and access with a DOI name for each data supplement.
- Pre-publication, password protected access to data supplements for journal editors in support to the peer-review process.
- Minimum formatting requirements from the authors.
- Direct communication with authors regarding changes to data and metadata, e.g. unit conversions, taxonomy updates.
- Open access to data in several widely accepted machine-readable formats.
- Creative Commons Attribution license which requires that author(s) are referenced if the data are reused.



Data preparation and quality control. We will be in direct contact with you during preparation and archiving of your data – [hide more...](#)

The PANGAEA data editorial ensures the integrity and authenticity of your data. Data might be submitted in the authors format and will be converted to the final import and publication format. The PANGAEA editors will check the completeness and consistency of metadata and data. Our editors are scientists from the earth and life sciences. We may identify potential problems with your data (e.g. outliers). Nevertheless, we will only take full responsibility for the technical quality. You will be responsible for the scientific quality of your data (e.g. the validity of used methods). After data have been archived you will receive a DOI name and you are requested to proof-read before the final version is published. In case your data are supplementary to a journal article you might reference the data in the article. In addition our web services allow for embedding data references dynamically on the article splash page ([example](#)). In case there is a moratorium on your data you can ask for access constraints.



Costs. PANGAEA can be used free of charge. Nevertheless, we appreciate any possible financial support – [hide more...](#)

PANGAEA is archiving and publishing data according to the Open Access Model. Basic operation is covered through public funding, but we have to seek for additional funds, in particular for preparing and archiving new data. In case data are submitted through a project which has publication costs as part of the funding, PANGAEA would appreciate a financial contribution of in average 300.– € for a data submission. Other forms/amounts of data contributions can be negotiated.

When you start the data submission process below, you will be redirected to the PANGAEA issue tracker that will assist you in providing metadata and uploading data files. Any communication with our editors will go through this issue tracker.

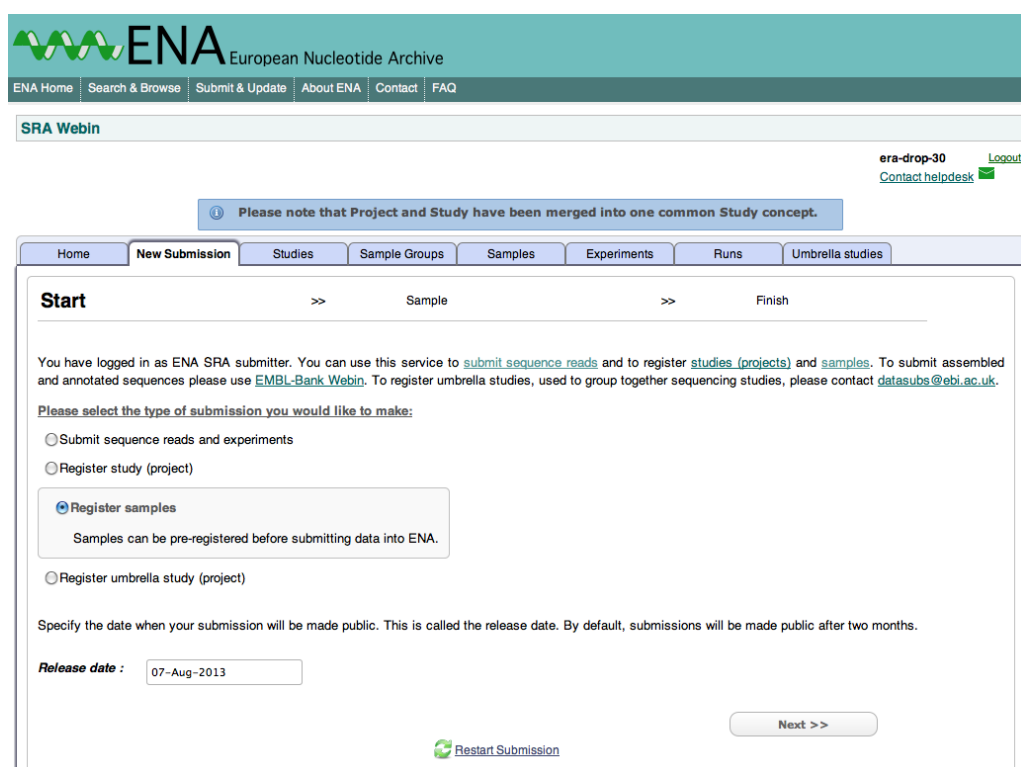
[SUBMIT YOUR DATA](#)

9. Submitting Sample Metadata to ENA

Each OSD marine research Cruise or Station needs to submit their OSD sample metadata, i.e. the MANDATORY MICRO B3 CHECKLIST for each OSD sample, to the ENA.

When molecular data of OSD samples become available from Sequencing Centre(s) these data will be linked to the submitted OSD sample metadata and integrated to the Micro B3 IS.

OSD sample metadata shall be submitted to the ENA using the WEBIN submission tool, <https://www.ebi.ac.uk/ena/submit/sra/#home>, designed for small-scale submissions of sequence data and metadata.



The screenshot shows the ENA SRA WebIn submission tool interface. At the top, there is a navigation bar with links for ENA Home, Search & Browse, Submit & Update, About ENA, Contact, and FAQ. Below this is a header for 'SRA WebIn' with a user status 'era-drop-30' and a 'Logout' link. A blue notification box states: 'Please note that Project and Study have been merged into one common Study concept.' The main content area has a breadcrumb trail: 'Home > New Submission > Studies > Sample Groups > Samples > Experiments > Runs > Umbrella studies'. The 'Start' section is active, showing a progress bar from 'Start' to 'Sample' to 'Finish'. The text explains that the user is logged in as an ENA SRA submitter and provides instructions on how to use the service. It asks the user to select the type of submission: 'Submit sequence reads and experiments', 'Register study (project)', 'Register samples' (which is selected), and 'Register umbrella study (project)'. A note states: 'Samples can be pre-registered before submitting data into ENA.' Below this, there is a field for 'Release date' set to '07-Aug-2013'. At the bottom, there is a 'Restart Submission' button and a 'Next >>' button.

Registration to the ENA's client system is required and may be in the future streamlined with the OSD registration process.

Please contact datasubs@ebi.ac.uk to create a new submission account. You can re-use this account for your any future submissions. Important: please state in the account request that you are an *OSD sampling Site*.

Upon registration you can login into the WEBIN submission system using received login details.



Please take the following steps to submit the MANDATORY MICRO B3 CHECKLIST for each OSD sample to the ENA:

1. Select the <New Submission> tab.
2. Select the option button <Register samples> and specify a release date of your sample metadata.
3. Navigate to checklists using the <Next> button.
4. Select the <ENA Micro B3> checklist from the menu of checklists. Note, this page will allow you to upload a completed spreadsheet from a previous session using the <Upload Spreadsheet> button.
5. Go to the next page. The right-hand side of this page lists all mandatory fields of the <ENA Micro B3> checklist. Additional optional fields can be added by expanding the button <Attributes> and selecting appropriate optional attributes. Selected optional attributes will appear on the right-hand side of this page.
6. Complete information on the right-hand side of this page, which is common to all your OSD samples.
7. In the section <Organism Details> type „marine metagenome“ in the <Search> field. This will automatically add an NCBI Taxonomy identifier <Tax Id> to your sample records.
8. Go to the next page and specify a number of your OSD samples using the <Add> button.
9. Complete all fields of this page, which contain sample-specific information. You have two options:
 - a. add info to each sample directly in the web interface and when completed submit your OSD samples with the <Submit> button.
 - b. download a spreadsheet to your local hard disk with the <Save Spreadsheet> button. This spreadsheet will contain all mandatory and selected optional fields. At your convenience complete all information locally and then start a new session (i.e. login again into WEBIN), repeat steps 1-4 and in the step 4 upload your spreadsheet using the <Upload Spreadsheet> button.

Submitted OSD samples will receive ENA accession numbers that will be linked to reads/sequence data submitted by the Sequencing Centre(s).

10. Submitting Reads and Sequence Data to ENA

Guidelines for Sequencing Centres Performing Nucleotide Sequence Analysis of the OSD Samples

Extensive guidelines for data submission using either the WEBIN submission system (for small scale submissions) or using the REST submission tool (for large scale submissions) is available at

http://www.ebi.ac.uk/ena/about/submit_and_update

Minimal reporting standards for Sequencing Centres, summarized in the **Table 3**, are incorporated in the above mentioned submission tools.

For all questions and enquiries please contact datasubs@ebi.ac.uk.

Table 3: Micro B3 checklist of OSD mandatory (highlighted) and recommended information about SEQUENCING of a Sample.

Recommendation applies to Sequencing Centres processing OSD data.

Field	Description	Control vocabulary/format	Example
Centre details	Full name and Country of the Institute/Centre responsible for the sequencing.	Free text;	Welcome Trust Sanger Institute, UK
Centre acronym	Preferred acronym of the Institute/Centre	Free text;	WTSI
Laboratory name	Name of the Laboratory/Department within the Sequencing Institute/Centre	Free text;	Microb Sequencing Unit
Contact details	Contact details of the sequence data submitter.	Format: <LASTNAME>, <FirstName>, <Institution>, <email>	BROWN, Paul; pbrown@institute1.eu
Study object	Study alias of relevant Study, issued by ENA, under which the sequencing experiment has been performed.	NA	<u>SRP001108</u>
Sample object	Sample alias of relevant registered sample, issued by ENA, for which the sequencing experiment has been performed.	NA	<u>SRS004892</u>
Instrument model	Model of the instrument used for the sequencing.	Term list ; *	454 GS FLX Titanium
Library strategy	Sequencing technique intended for this library.	Term list ; *	amplicon

Library source	The type of source material that is being sequenced.	Term list ; *	metagenomic
Library selection	Specifies whether any method was used to select for or against, enrich, or screen the material being sequenced.	Term list ; *	PCR
Raw sequence data files	File(s) containing reads.	Term list ; *	wco2.sffl
Sequencing Experiment name	Unique name for the Sequencing Experiment.	Free text;	454 GS FLX sequencing; SSU rRNA Gene Hypervariable Region Tag Sequencing (Bacterial V6)
Design description	Details about the setup and goals of the sequencing experiment.	Free text;	The V6 hyper-variable region of the 16S rRNA gene was amplified using pyrosequencing to investigate intra-annual microbial diversity over 11 months during 2007 at the surface of the L4 sampling site in the Western English Channel.
Library name	User name of the library created.	Free text;	BacV6: 967F – 1046R
Library construction protocol	Details on- or a link to a literature, reference, electronic resource or a standard operating procedure (SOP) of nucleic acid extraction, amplification method and PCR primers.	Free text;	Example at http://www.ebi.ac.uk/ena/data/search?query=SRX008290
MD5 checksum	MD5 checksum for each data file	Hexadecimal number, 32 digits long	e4d909c290d0fb1ca068ffaddf22cbd0

* Term list for this attribute is available from the ENA submission system. (http://www.ebi.ac.uk/ena/about/sra_submissions).

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Not *et al.* (in preparation)





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Annex I OSD Logsheets

SAMPLING	 	SAMPLING_Campaign :		
		SAMPLING_Site :		
		SAMPLING_Platform :		
	SAMPLING_Authors :		Last name	First name

EVENT	EVENT_Date/Time		yyyy	mm	dd	hh	mm	ss	UTC
			Start						
			End						
			+N/ -S	dd.0000			+E/-W	ddd.0000	
	EVENT_Lat/Long		Start						
			End						
	EVENT_Device :								
EVENT_Method :									
EVENT_Comment :									

ENVIRONMENT	ENVIRONMENT_Marine_Region		Term Example: Northern Baltic	
	ENVIRONMENT_Biome		 Term Example: marine pelagic biome	
	ENVIRONMENT_Feature		Term Example: marine wind mixed layer	
	ENVIRONMENT_Material		 Term Example: sea water	
	ENVIRONMENT_Other Parameters than Temperature & Salinity:	() No () Yes, please use additional sheet to provide details		



COMMENT

SAMPLING		SAMPLING_Campaign :		EVENT_Date/Time_Start :	
		SAMPLING_Site :		EVENT_Latitude_Start :	
		SAMPLING_Platform :		EVENT_Longitude_Start :	

		Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
SAMPLES	SAMPLE_ID :	Affix Barcode	Affix Barcode	Affix Barcode	Affix Barcode	Affix Barcode	Affix Barcode
	SAMPLE_Protocol_Label :						
	SAMPLE_Depth (m):						
	SAMPLE_Quantity :						
	SAMPLE_Container :						
	SAMPLE_Content :						
	SAMPLE_Size-Fraction_Upper-Threshold :						
	SAMPLE_Size-Fraction_Lower-Threshold :						
	SAMPLE_Treatment_Chemical :						



	SAMPLE_Treatment_ Storage :						
ENVIRONMENT	ENVIRONMENT_ Temperature (deg C)						
	ENVIRONMENT_ Salinity (PSU)						
	ENVIRONMENT_ 						



ENVIRONMENTAL PARAMETERS	CATEGORY	CHECK if done	PARAMETER	DESCRIPTION	Control vocabulary/format *	
	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 Optical			Downward PAR	Visible waveband radiance and irradiance measurements in the water column	SDN:P02:75:VSRW SDN:P06:46:UMES for µE/m ² /s	



Properties		Turbidity	Transmittance and attenuation 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:UGPL 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/Microp plankton	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/Macrop plankton	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
		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>)





Annex II

a) Material Transfer Agreement (MTA) - Agreement between OSD Participant and Argonne National Laboratory

Agreement on the Transfer of Marine Microorganisms from Recipient 1 (OSD Institution) to Recipient 2 (Argonne National Laboratory)

THIS AGREEMENT is made on _____ [insert date]

BETWEEN:

OSD Sampling Institution in,

[Insert the name of Recipient 1 /the OSD institution and its representative and the full contact details]

(“the Transferor”)

AND:

Argonne National
Laboratory _____

[Insert the name of the Recipient 2 / Sequencing institution and its 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 Argonne National Laboratory of samples of marine microorganisms accessed in the framework of the Micro B3 project. It shall ensure that the Argonne National Laboratory endorses the Micro B3 Agreement on Access to Marine Microorganisms and Benefit Sharing 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 shall bear all the costs incurred in delivering the genetic resources.

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 concluded between the Transferor and the competent national authority of as indicated by its national focal point to the CBD (the provider of the GR in the original agreement).

2.2 The said agreement is attached to the present agreement.

(Location, Date)

(Transferor)

(Transferee)

b) Data Transfer Agreement (DTA) - Agreement between the Argonne National Laboratory and the European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI)

Agreement on the Transfer of Data from Recipient 2 (Argonne National Laboratory) to Recipient 4 (European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI))

THIS AGREEMENT is made on _____ [insert date]

BETWEEN:

Argonne National Laboratory

[Insert the name of the Recipient 2 and its representative and the full contact details]
("the Transferor")

AND:

European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI)

[Insert the name of the Recipient 4 / bio-archiving Institution and its 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 the Argonne National Laboratory of data extracted from marine microorganisms accessed in the framework of the Micro B3 project. It shall ensure that the EMBL-EBI endorses the Micro B3 Agreement on Access to Marine Microorganisms and Benefit Sharing concluded between the OSD sampling Institution and the competent authority, as appropriate.

The Parties to this agreement hereby agree as follows:

Article 1 Transfer of Genetic Resources

1.3 The Transferor will deliver to the Transferee data extracted from marine microorganisms accessed in the framework of the Micro B3 project.

Article 2 VIRAL CLAUSE

2.3 The Transferee endorses the provisions specified in article 5.2 of the Micro B3 Agreement on Access to Marine Microorganisms and Benefit Sharing concluded between the OSD Institution in Italy and the competent national authority of Italy as indicated by the competent national authority of ... as indicated by its national focal point to the CBD (the provider of the GR in the original agreement).

2.4 The said agreement is attached to the present agreement.

(Location, Date)

(Transferor)

(Transferee)

Annex III

Draft Agreement between OSD Participant and SI NMNH

**Agreement
Between
MICRO B3 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 MICRO B3 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 MICRO B3 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 pilot 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.

III. Scope

This Agreement covers Collections and associated information from MICRO B3 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 from the Argonne National Labs. The number of samples included within each portion sent to the SI NMNH Biorepository will be determined by MICRO B3 OSD Participants and Argonne National Labs.

IV. Terms

SI NMNH will:

- A. Cold preserve up to 10,000 environmental samples at -80 C collected on or after July 21 2014 and OSD pilot collecting events leading up to July 21 2014, provided they comply with the OSD Bioarchiving pre-shipping checklist and meet the SI NMNH data standards. Expose information on primary OSD Collection data and provide access to this information for the duration of the loan.



B. Provide access to the collection upon request following the terms outlined in the Scientist-provider agreement (under Micro B3) for the duration of the loan.

C.

Micro B3 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 NMNH with all permits related to collecting and transporting the OSD Collections.

E. Provide NMNH with a copy of the Scientist Provider Agreement (under Micro B3).

F. Abide by all applicable laws and regulations, including appropriate access and benefits sharing.

G. Provide NMNH with metadata compliant with the SI NMNH data standards.

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 from in situ sources), and SI NMNH will reevaluate this agreement and the need for SI NMNH to retain custodial responsibilities of the OSD collection.

VI. Agreed

MICRO B3 OSD Participant

By

Contact name

SI NMNH

By

Contact name



Annex IV

Model Agreement on Access to Marine Microorganisms and Benefit Sharing

MICRO B3 Agreement on Access to Marine Microorganisms and Benefit Sharing

Result of MICRO B3 WP8

<http://www.microb3.eu/>

Version 1.0. of 10th June

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Acknowledgements

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Agreement on Access to Marine Microorganisms and Benefit Sharing

Annotated

(version of 10.06.2013)

THIS AGREEMENT is made

BETWEEN:

[Insert the name of the Provider State institution² and its representative and the full contact details]

("the Provider")

AND:

[Insert the name of the Recipient institution³ 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

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.

- to develop innovative bioinformatic 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 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,

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 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 shall be set out in a material transfer agreement, and agreeing that this shall be included in the present 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

1.4 The agreement sets out the terms for the access to genetic resources found in/on the Provider State's 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.

1.5 The agreement is part of the Micro B3 Consortium Agreement⁴. Its rights and obligations extend to all Micro B3 partners.

1.6 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.
- 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 which is 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 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.

⁴ The Consortium Agreement is publicly accessible at the MicroB3 website www.microb3.eu



- 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 samples⁵, including the kind of genetic resources⁶ included, if known:

- b.) Number and quantity of samples:

- c.) Geographical location of collection⁷:

- 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.

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.



(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 resources⁹.

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

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:

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.



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

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.



- 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]:
-

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, Pangea 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.

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 knowledge¹¹.

Article 9 SHARING OF KNOWLEDGE

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 necessary¹²: _____

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

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.

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



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 of copies) 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:

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.

_____ higher upfront benefit sharing under article 11 as a *quid pro quo* for crossing this article.

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.



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.

(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, 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)