

Project	AtlantOS – 633211
Deliverable number	6.5
Deliverable title	Metrology reference and standard materials
Description	Reference material for trace elements linked to the International GEOTRACES programme (GEOMAR and UOP), create genomic standards and organize their community analysis (Ribocon), and standardize DNA extraction and sequencing (Ribocon and AWI).
Work Package number	6
Work Package title	Cross-cutting issues and emerging networks
Lead beneficiary	GEOMAR
Lead authors	<p>GEOMAR: Eric Achterberg</p> <p>AWI: Pier L. Buttigieg, Felix Janssen</p> <p>Ribocon: Jörg Peplies</p> <p>UOP: Paul Worsfold, Maeve Lohan, Simon Ussher</p>
Contributors	
Submission data	16/08/2018 (delayed due to vacation time)
Due date	31/07/2018
Comments	<p>Reference material for trace elements has been produced and is being distributed.</p> <p>Genomic standards and community analysis have been conducted. Best Practice for standardised DNA extraction protocols and sequencing procedures has been developed</p>



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement n° 633211.

Stakeholder engagement relating to this task*

WHO are your most important stakeholders?	<input type="checkbox"/> v Private company If yes, is it an SME <input type="checkbox"/> v or a large company <input type="checkbox"/> ? <input type="checkbox"/> v National governmental body <input type="checkbox"/> v International organization <input type="checkbox"/> NGO <input type="checkbox"/> v others Please give the name(s) of the stakeholder(s): RIBOCON, various scientists from multiple countries
WHERE is/are the company(ies) or organization(s) from?	<input type="checkbox"/> v Your own country <input type="checkbox"/> Another country in the EU <input type="checkbox"/> Another country outside the EU Please name the country(ies): ...Germany
Is this deliverable a success story? If yes, why? If not, why?	<input type="checkbox"/> Yes, because successful workshops have been held, with strong engagement of stakeholders, and these were linked to the development of reference materials and creation of Best Practice documents. The reference materials for trace elements have been around for ca. 8 years (but have become scarce and needed to be produced again), but the development of genomic reference materials is ground-breaking work and the team has made tremendous efforts to get this organised and distributed. <input type="checkbox"/> No, because
Will this deliverable be used? If yes, who will use it? If not, why will it not be used?	<input type="checkbox"/> Yes, the outputs of the deliverable will be used by companies, ocean observing organisations and individual researchers. The community building efforts, the outputs of the meetings, and the capacity building through the development of trace element and genomic reference materials will be of great importance to the observing communities. <input type="checkbox"/> No, because

NOTE: This information is being collected for the following purposes:

1. To make a list of all companies/organizations with which AtlantOS partners have had contact. This is important to demonstrate the extent of industry and public-sector collaboration in the obs community. Please note that we will only publish one aggregated list of companies and not mention specific partnerships.
2. To better report success stories from the AtlantOS community on how observing delivers concrete value to society.

*For ideas about relations with stakeholders you are invited to consult [D10.5](#) Best Practices in Stakeholder Engagement, Data Dissemination and Exploitation.

D6.5 Metrology reference and standard materials: Reference material for trace elements linked to the International GEOTRACES programme (GEOMAR and UOP), create genomic standards and organize their community analysis (Ribocon), and standardize DNA extraction and sequencing (Ribocon and AWI). PM40

Authors GEOMAR: Eric Achterberg

AWI: Pier L. Buttigieg, Felix Janssen, author Ribocon: Jörg Peplies

The collection and creation of reference seawater materials, in connection with the International GEOTRACES Programme, has been very successful. These materials are distributed free of charge to users and provide a backbone to high quality measurements of trace metals in seawater, allowing data to be comparable and of high quality.

Over 550 samples of 500 ml of deep seawater consensus material (from a depth of 1000 m) for trace metal were collected by GEOMAR on FS Meteor cruise M121 using trace metal clean conditions in the southeast Atlantic Ocean in December 2015. The samples have been assessed for stability, and subsequently shared with international partner laboratories. The samples will be distributed in autumn 2018 to the global community, at no cost to the users other than shipping costs. The exercise forms a collaboration between ATLANTOS and the International GEOTRACES Programme.

In a further activity, over 550 samples for GEOTRACES surface seawater consensus material for trace metals were collected by GEOMAR on FS Meteor cruise M145 in March 2018 in the equatorial Atlantic Ocean using trace metal clean conditions. The samples will be tested for stability and various experienced laboratories will undertake sample analysis for trace metals. Intention is to share with community in spring 2019.

Furthermore, over 500 samples for GEOTRACES deep seawater consensus material for trace metals were collected by GEOMAR on FS Meteor cruise M147 in April 2018 in the equatorial Atlantic Ocean using trace metal clean conditions. The samples will be tested for stability and various experienced laboratories will undertake sample analysis for trace metals. Intention is to share with community in spring 2019.

The consensus reference materials have been instrumental in enhancing data quality in the international trace metal community. The materials are distributed free of charge, and consequently there is no difficulty for laboratories in developing countries to obtain and use the materials. GEOMAR has obtained a sum of 1500 dollar from SCOR to pay for shipment of the materials to laboratories in third world countries.

In the global omics community, a comparison between different protocols by means of standard materials is novel and still an area of research. Discussions on feasible approaches at and following the AtlantOS 'workshop on enhancing interoperability & coordination of long-term omics observations' identified the need for a combination of genomic standards (i.e., controlled DNA mixes) and natural samples (i.e., frozen filters). This facilitates the development and testing of strategies for inter-observatory calibration that address the full pipeline from DNA extraction to sequencing and finally data analysis with bioinformatics tools. While genomic samples allow sequencing and bioinformatics protocols to specifically address under fully controlled conditions, the exchange of natural samples extends the scope towards effects of DNA extraction, primer choice and amplification conditions.

Through the GLOMICON community established at the workshop (see D6.4), AWI and Ribocon have partnered with the Monterey Bay Aquarium Research Institute (MBARI) to create and exchange genomic standards and natural samples (more detailed information on the material is found below). This will serve to assess the impact of varying procedures across observatories on raw sequencing results and the biodiversity information obtained and represents a major step towards standardization of methods from DNA extraction to data analysis. The recipes for genomic standards and sampling protocols are made available to the GLOMICON community (as a shared living online protocol¹) and all community members are invited to participate in the intercalibration. The genomic standard materials themselves as well as environmental sample material collected during the summer season of 2018 are made available by mid-August 2018. Extraction and sequencing by AWI, MBARI, and additional participating parties is expected to be completed by mid-Autumn 2018. Ribocon is supporting the coordination of these activities by providing the prototype of a dedicated online platform to systematically record and monitor data and information flows (including procedural records) of the GLOMICON community. The platform will provide pre-defined data schemes allowing users to register projects in a fully standardized manner (including support for standardized metadata recording) and will facilitate centralized access to omics raw data and corresponding metadata, as well as analysis results for data intercomparison. This is done by linking up with existing infrastructures such as ENA or PANGEA where users need to archive their actual (meta)data. The platform is intended as an initial case study in preparation for a next generation GLOMICON coordination and harmonization web environment. Further, Ribocon provides a baseline bioinformatics analysis of the data based on the broadly used SILVAngs system, which can be compared to the partners' native workflows in aid of community-wide data product calibration/standardization. Collaboration with the German Federation for Biological Data ensures that the sample exchanges are generating data and (meta)data along existing community standards established by the Genomics Standards Consortium.

Genomic standard material

DNA mixes from Mock communities or unicellular eukaryotes of known species composition and relative gene copy numbers are prepared by AWI as amplicons. 18S genes from DNA extracts obtained from a total of 22 cultures of unicellular algae including the most important planktonic primary producers are amplified separately. DNA concentration/copies per volume are determined for the individual PCR extracts, and mixed to obtain DNA mixes with a known concentration of 18S gene copy numbers. This allows a quantitative comparison to the relative abundances of sequencing reads. Sets of 5 replicates each are prepared for analysis at MBARI, AWI, and other participating parties. In order to address compositionality effects two different mixes are prepared, (1) an 'evenMock' with even gene copy numbers for all species, and (2) a 'bloomMock' where copy numbers are chosen to mimic a bloom situation with a combination of dominant and rare types. Table 1 shows the different taxa and their relative contribution to the total copy number in case of the 'evenMock' and 'bloomMock' DNA mixes.

Table 1: List of taxa represented in the genomic standard material and their share in the overall composition in terms of % of total copy numbers in case of the 'evenMock' and 'bloomMock' standards.

Taxon	Taxonomic group	% of total copy numbers	
		evenMock	bloomMock
<i>Micromonas pusilla</i>	Chlorophytes	4.5	10.0
<i>Prasinococcus capsulatus</i>	Chlorophytes	4.5	0.5

¹ <https://docs.google.com/document/d/1U6RZnmgDodXqhp22wMmmejucFLW41yynITawZw7PyJ0>

<i>Pyramimonas parkeae</i>	Chlorophytes	4.5	2.0
<i>Prasinoderma colonial</i>	Chlorophytes	4.5	2.0
<i>Emiliana huxleyi</i>	Haptophytes	4.5	0.5
<i>Phaeocystis globose</i>	Haptophytes	4.5	2.0
<i>Phaeocystis pouchetii</i>	Haptophytes	4.5	35.0
<i>Chrysochromulina kappa</i>	Haptophytes	4.5	2.0
<i>Karlodinium veneficum</i>	Dinoflagellates	4.5	2.0
<i>Alexandrium minutum</i>	Dinoflagellates	4.5	0.5
<i>Heterocapsa triquetra</i>	Dinoflagellates	4.5	2.0
<i>Prorocentrum micans</i>	Dinoflagellates	4.5	10.0
<i>Pseudonitzschia sp.</i>	Diatoms	4.5	0.5
<i>Chaetoceros mülleri</i>	Diatoms	4.5	2.0
<i>Chaetoceros socialis</i>	Diatoms	4.5	2.0
<i>Odontella aurita</i>	Diatoms	4.5	2.0
<i>Thalassiosira sp.</i>	Diatoms	4.5	2.0
<i>Thalassiosira Hyalina</i>	Diatoms	4.5	2.0
<i>Thalassiosira weissflogii</i>	Diatoms	4.5	10.0
<i>Melosira arctica</i>	Diatoms	4.5	10.0
<i>Leptocylindrus danicus</i>	Diatoms	4.5	2.0
<i>Skeletonema costatum</i>	Diatoms	4.5	0.5

Shared environmental samples

MBARI and AWI provide natural samples from surface waters collected in the western North Pacific and the Arctic Fram Strait, respectively. MBARI's samples originate from the MBARI time-series station at the shelf break off Davenport while AWI's samples are taken at the LTER observatory HAUSGARTEN as part of the FRAM observatory infrastructure west of Svalbard. Water from several simultaneously closed Niskin bottles is combined in a common carboy and aliquots are filtered through 0.2 µm disc filters (Fig. 1) and immediately frozen. Filters are exchanged among participants for DNA extraction and analysis of natural communities of eukaryotic organisms. The material can also be used for comparisons of protocols addressing bacterial and archaeal communities.

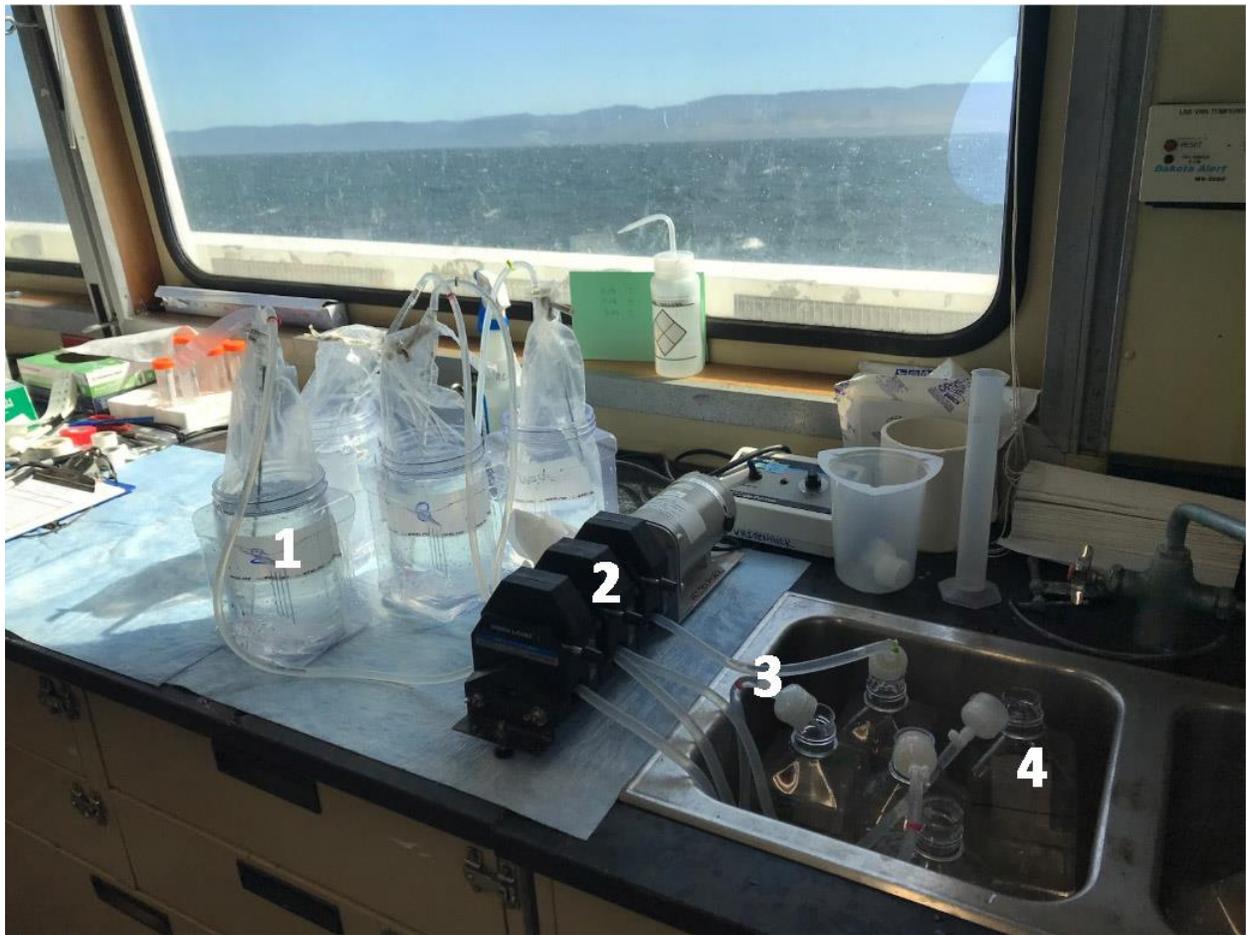


Fig. 1: Shipboard filtration of surface waters collected in the western Pacific Ocean. The sample intakes are inserted into sterile whirlpak bags (1) or a common carboy. The water is transported by a peristaltic pump system (2) and passed through the filter that is inserted into a sterile multi-use cartridge (3). The total volume filtered is determined from collecting vessels (4) at the outflow (Image: Kathleen Pitz, MBARI).