



BlueTools

Initial contours for DNA Extraction protocols



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Technical References

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Project Title	INNOVATIVE TOOLS FOR SUSTAINABLE EXPLORATION OF MARINE MICROBIOMES: TOWARDS A CIRCULAR BLUE BIOECONOMY AND HEALTHIER MARINE ENVIRONMENTS
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Lead beneficiary	UA
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V2			
V3			

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1. Introduction:

Standards are the key to accelerating science, avoiding redundancy and enabling the integration of data streams. As part of the Work Package 2, milestone MS3, this study provides the initial contours for responsible standardization in sampling, storage, sample pretreatments and DNA extraction. Subsequently, the consortium will optimize and propose its own modifications, releasing the updated standards and protocols in the deliverable D2.1 due on Month 6.

2. Outlook and initial contours for standardization in BLUETOOLS:

After reviewing the most important projects that have been involved in the standardization of steps along the metagenome analysis workflow, from sampling to DNA extraction, the most relevant aspects of each have been extracted, in order to assess which SOPs/Procedures would be useful as foundations for SOPs developed within BLUETOOLS (Table 1). Given the early placement of this task, where no consensus decisions on SOPs for the different parts of the metagenomics workflow can be reached, the SOPs of the project MetaFluidics (GA-685474) are used as a starting point. Optimization and alternatives will be proposed and evaluated, given the peculiarities of the biomes to be analyzed within BIOTOOLS, and the corresponding results will be incorporated in deliverable D2.1 due on month 6.

MS3 – SOPs for DNA extraction

Table 1. Applicable SOPs/Procedures for BLUETOOLS. SOPs provided by standardization projects are indicated by using the project acronym (along with URL or reference when provided). Rest of procedures have been supplied from literature search or BLUETOOLS partners' expertise/input. NP stands for non-provided

Nucleic extraction workflow step	Water (Fresh/Ground/Marine)	Brine water/Salterns	Hot springs	Sediments	Ascidians/Posidonia
Metadata	<p>GSC: MixS (MIMS) (https://genomicsstandardsconsortium.github.io/mixs/MIMS/)</p>				
Sampling	<p>Micro B3: Ocean Sampling Day Handbook Gilbert <i>et al.</i>, 2010a</p>	<p>Gomariz <i>et al.</i> 2014 Ramos-Barbero <i>et al.</i> 2022</p>	<p>Jiménez <i>et al.</i>, 2012 López-López <i>et al.</i>, 2015</p>	<ul style="list-style-type: none"> EMP500 (http://press.igsb.anl.gov/earthmicrobiome/emp500/emp500-sample-submission-guide/) 	<ul style="list-style-type: none"> Martínez-García <i>et al.</i> 2008(doi:10.1111/j.1462-2920.2008.01761.x) Rubio-Portillo <i>et al.</i> 2021(doi.org/10.1128/mSystems.00866-21.)
Sample labelling	<p>Micro B3: OSD Sampling Protocol for Prokaryotes (Ocean Sampling Day Handbook (http://www.assembleplus.eu/sites/assembleplus.eu/files/public/manual/OSD_Handbook_2016.pdf))</p>			<ul style="list-style-type: none"> EMP500 (http://press.igsb.anl.gov/earthmicrobiome/emp500/emp500-sample-submission-guide/) 	<p>Micro B3: OSD Sampling Protocol for Prokaryotes</p>
Sample preservation					
Storage container and conditions	<p>Plastic bottle (-80°C) +RNAlater® If needed Falcon™ tube For viable cells store at 4 °C</p>				
Sample pretreatment	<p>Micro B3: OSD Sampling Protocol for Prokaryotes (Ocean Sampling Day Handbook)</p>	<p>XMP: CTAB (Omega Bio-tek Inc) with enzymatic digestion (MAC4L polyzyme mix) and sapphire beater beads (http://extrememicrobiome.org)</p>	<p>Jiménez <i>et al.</i>, 2012 López-López <i>et al.</i>, 2015</p>	<p>No pretreatments or alternatively sediment particle removal by sedimentation and/or centrifugation.</p>	<p>Washing to remove debris</p>

MS3 – SOPs for DNA extraction

	(https://www.microb3.eu/sites/default/files/osd/OSD_Handbook_June_2015.pdf)	g/methods/#1453529639867-2ea70634-a507		
		Santos <i>et al.</i> , 2010 Filtration for enrichment (0.22µm Millipore filter)		
DNA extraction	<ul style="list-style-type: none"> • Micro B3: Ocean Sampling Day Handbook. Gilbert <i>et al.</i>, 2010a • AllPrep Bacterial/Fungal DNA/RNA/Protein Kit QIAGEN 	<ul style="list-style-type: none"> • XMP: Phenol +MoBio PowerWater® DNA Isolation Kit • Agarose plug extraction (Santos <i>et al.</i>, 2010) • Ultrasens Kit Epicentre® • CTBA method (Mirete <i>et al.</i>, 2015) • Qbiogene BIO101 FastDNA® Spin kit for soil • MoBio PowerMag® Soil DNA Isolation Kit • PowerSoi®L-htp 96 Well Soil DNA Isolation Kit 		<ul style="list-style-type: none"> • Agarose plug extraction (Santos <i>et al.</i>, 2010) • Ultrasens Kit Epicentre® • CTBA method (Mirete <i>et al.</i>, 2015) • Qbiogene BIO101 FastDNA® Spin kit for soil • MoBio PowerMag® Soil DNA Isolation Kit • PowerSoi®L-htp 96 Well Soil DNA Isolation Kit
DNA purification	<ul style="list-style-type: none"> • Micro B3: Ocean Sampling Day Handbook Gilbert <i>et al.</i>, 2010a 	Díez <i>et al.</i> , 2010		
DNA preservation	<ul style="list-style-type: none"> • Micro B3: OSD Sampling Protocol for Prokaryotes (Ocean Sampling Day Handbook) (http://www.assembleplus.eu/sites/assembleplus.eu/files/public/manual/OS) 	<ul style="list-style-type: none"> • EMP: Norgen Biotek Corp. (http://www.ghbn.org/) Polysciences (http://www.polysciences.com/) DNA Genotek (http://www.dnagenotek.com/) DMSO special mixes Ethanol special mixes • -20oC or -80oC 		-20°C or -80°C

MS3 – SOPs for DNA extraction

	D_Handbook_2016.pdf			
Quality check and quantification	<ul style="list-style-type: none"> • Gel electrophoresis size assignment • Spectrophotometric analysis: A260/280, A260/230 • Qubit • Bionalyzer 			
DNA Amplification	Ilmberger <i>et al.</i> , 2014	<ul style="list-style-type: none"> • EMP: 16S rRNA 18S rRNA 	<ul style="list-style-type: none"> • EMP: 16S rRNA 18S rRNA 	
Shipment of samples	<ul style="list-style-type: none"> • Micro B3: OSD Sampling Protocol for Prokaryotes (Ocean Sampling Day Handbook) (http://www.assembleplus.eu/sites/assembleplus.eu/files/public/manual/OSD_Handbook_2016.pdf) 		<ul style="list-style-type: none"> • EMP: (http://www.earthmicrobiome.org/emp-standard-protocols/shipping/) 	