



Jan 28, 2025

Culturing and Long-term Preservation of Cyanobacterial Communities from Environmental Samples

DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvr97qzvmk/v1>

Sarah Duxbury^{1,2}, Alberto Scarampi¹, Mary Coates¹, Orkun S. Soyer¹

¹School of Life Sciences, Gibbet Hill Campus, University of Warwick, CV4 7AL, UK;

²University of Exeter, Science and Engineering Research Support Facility (SERSF), Penryn, Cornwall, TR10 9FE, UK

OSS Lab



Mary Coates

University of Warwick

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.6qpvr97qzvmk/v1>

Protocol Citation: Sarah Duxbury, Alberto Scarampi, Mary Coates, Orkun S. Soyer 2025. Culturing and Long-term Preservation of Cyanobacterial Communities from Environmental Samples. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.6qpvr97qzvmk/v1>



License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: January 09, 2025

Last Modified: January 28, 2025

Protocol Integer ID: 118458

Keywords: Biofilm, Cryopreservation, Metagenomic analysis , term preservation of cyanobacterial community, cyanobacterial community, environmental sample, environmental samples this protocol, term preservation

Funders Acknowledgements:

Gordon and Betty Moore Foundation

Grant ID: GBMF9200

Abstract

This protocol describes the culturing and long-term preservation of cyanobacterial communities from environmental Samples.

Guidelines

Background

This protocol follows on from protocol 'Environmental Sampling of Freshwater Microbial Communities' and is for the establishment of laboratory enrichment cultures of cyanobacterial communities and their long term preservation. We have successfully used this protocol to enrich cyanobacterial communities, for example:

<https://www.biorxiv.org/content/10.1101/2022.12.13.520286v2>

Materials

Media:

Media:

- We mostly use BG11+ media according to the DSMZ protocol:

https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1593.pdf, (**Figure 1**) omitting Vitamin B12, instead, add a vitamin mix supplement (**Figure 2: OSS Lab protocol OSM02_02**).

Note

We have also successfully used BG11+ without vitamin mix, or even BG11 (which does not have Vitamin B12).



1593. Cyanobacteria Medium BG11+

Minerals	Volume [ml/l]	Stock Solutions [g/l]
Mg(SO ₄) x 7H ₂ O	10	7.5
CaCl ₂ x 2H ₂ O	10	3.6
Na ₃ -citrate x 2H ₂ O	10	0.6
Na ₂ -EDTA x 2H ₂ O	10	0.1
Trace metal mix	1.0	-

Adjust to 1000 ml with H₂O dist. and autoclave.

After cooling, add the following filter sterilized components

Minerals	Volume [ml/l]	Stock Solutions [g/l]
NaNO ₃	5.0	300
BG11-Mix	2.0	-
Vitamine B12	1.0	0.02

	[g/l]
BG11-Mix	
K ₂ HPO ₄ x 3H ₂ O	20
Na ₂ CO ₃	10
Fe-NH ₄ -citrate	3.0

		[g/l]	[ml/l]	
Trace metal Mix	H ₃ BO ₃	2.9	<-	
	MnCl ₂ x 4H ₂ O	1.81	<-	
	ZnSO ₄ x 7H ₂ O	0.22	<-	
	Na ₂ MoO ₄ x 2H ₂ O	0.39	<-	
	CuSO ₄ x 5H ₂ O	->	10 ml	0.8 g/100 ml Stock Sol.
	Co(NO ₃) ₂ x 6H ₂ O	->	10 ml	0.5 g/100ml Stock Sol.

© 2017 DSMZ GmbH - All rights reserved

Figure 1: BG11+ from DSMZ



A	B
Vitamins	g/L(stock)
Biotin	0.020
Folic Acid	0.020
Pyridoxin HCl	0.100
Thiamine HCl	0.050
Riboflavin	0.050
Nicotinic Acid	0.050
D-Ca-Pantothenate	0.050
p-Aminobenzoic Acid	0.050
Vitamin B12	0.001
Lipoic Acid	0.050

Figure 2: Vitamin mix from OSS Lab media sheet OSM02_02



Establishing Lab Cultures

- 1 Water samples, usually from freshwater in our case, place with or without visible granules or biofilm (referred to as '*original sample*') in media using a 50:50 dilution and left to establish for at least 35 days (referred to as '*Passage 0*').
- 2 You can use glass Erlenmeyer flasks, glass bottles, medical flasks, or plastic culture flasks. Seal the culture vessels with gas permeable membranes to create an aerobic environment or with "breathable" lids.

Note

It is also possible to use sealed vessels as per this paper:
<https://pubmed.ncbi.nlm.nih.gov/34740965/>

Routine Sub-culturing

- 3 Once established, we usually sub-culture every 35 to 49 days using a 1:50 dilution (referred to as '*Passage 1*', 2 ...etc).

Note

That sub-culturing process – together with the media used - is expected to cause a loss in community diversity through "species sorting" and/or "evolution". In this context, sub-culturing time would likely affect community assembly dynamics, e.g. shorter durations selecting more for faster-growing species. Dilution level would also affect community assembly, e.g. lower dilution factor would be expected to enable rare/slow-growing species to persist. See this paper that explored the duration aspect to some extent – under glucose-supplemented conditions
(<https://www.sciencedirect.com/science/article/pii/S2589004223009562>).

Growth Conditions

- 4 Grow cultures under continuous 12h/12h light/dark cycles with white, fluorescent, or *LED illumination of  20 μL -  50 μL photons $\text{m}^{-2} \text{s}^{-1}$ at  Room temperature under static conditions.



**we are currently using URBAN BUDDY 240w LED Quantum Board - Veg Spec as light source.*

Note

That light conditions, both in terms of intensity, spectra, or duration can be altered to suit the experimental goals/limitations.

Cryopreservation – Long Term Storage

20m

- 5 We successfully use 10% glycerol in media of choice (e.g., BG11+ vitamin mix) as a cryoprotectant.

Note

Note that we were also able to preserve cultures long term using 10% v/v DMSO and 5% v/v methanol in media, but due to associated hazards with these chemicals we choose to work with 10% glycerol.

- 6 Prepare 10% v/v ( 10 mL /  100 mL) glycerol in distilled water and autoclave, leaving out volume for later addition of sterile medium components.

- 7 Aliquot  1 mL of culture from each flask into a sterile 2 ml screw-cap tube.
Centrifuge at  10000 x g, 00:05:00 and discard the supernatant by pipetting.

5m

- 8 Add  1 mL of the cryoprotectant media to the cell pellet and re-suspend cells (by pipetting).



- 9 Leave the tubes for a  00:15:00 incubation period at low light intensity ($<5 \mu\text{mol m}^{-2} \text{s}^{-1}$). This serves as an equilibration period to protect the cells from cryoprotectant damage.

15m

- 10 Store long term in a  -80°C freezer.



Cryopreservation - Revival of Cryostocks

1d 0h 5m

11

Note

Remove cryostock tube from the -80 °C freezer and leave to defrost at Room temperature , until the required volume can be sampled (e.g., we sample between 150 µL and 1 mL , depending on the culture density and/or distribution of biofilm material within the cryostock).

12

Transfer the thawed sample to a 1.5 ml sterile microcentrifuge tube, and immediately centrifuge at 6500 x g, 00:05:00 (so to reduce time on cryoprotectant, which is considered toxic) (Rastoll 2013)). Discard supernatant and add 1.5 mL of fresh culture medium (e.g., BG11+ vitamin mix), without cryoprotectant, to wash the cells. Perform this centrifugation and washing step twice in total then re-suspend the cells in an equal volume of fresh media.

5m



13

Maintain the culture aliquots at Room temperature in the dark (e.g., in a cupboard) for 24:00:00 , so to reduce the light intensity exposed to the cells after thawing (to protect against cell damage) as suggested in Day (2007)). This step is likely only to be required for cultures containing photosynthetic bacteria, rather than purely (heterotrophic) bacterial isolates. We have also had success reviving cultures without this step.

1d

14

After dark incubation, place the culture in fresh media (e.g., 300 µL of revived culture in 14.7 mL of media in a 50 ml Erlenmeyer flask). This represents a 1:50 dilution of the culture aliquot. We usually don't set up replicate cultures at this step, but create replicates at the point of 1st subculturing.

- Culture under optimal growth conditions, i.e., ~ 20 µL - 50 µL $\text{m}^{-2} \text{s}^{-1}$ light intensity over a period of time to allow sufficient culture re-growth.

Culture Pellets for DNA Extraction: make 2 pellets per culture after 49 days growth.

10m



- 15 Pre-weigh sterile 2 ml screw-cap tubes on a mg weighing balance and record the weights of the empty tubes.
- 16 For liquid/suspension cultures, shake the flask vigorously so that culture is as well-mixed as possible and then aliquot  1.5 mL volume of culture sample into a pre-weighed screw-cap tube.
- For cultures with spatial organization (e.g., biofilms, granules, aggregates, etc.), biofilm material can be added to the screw-cap tube up to approximately the 0.25 ml mark on the tube. If pipetting large aggregates, use cut-end P1000 tips, or suction onto the tip of a serological pipette as necessary. Depending on the enrichment conditions (e.g., nitrates omitted in the growth medium), the biofilms may stick very strongly on the inner walls of the culture flasks. In this case, use a sterile inoculation loop to detach the cell material from the flask and mix it in the solution.
- 17 Centrifuge the tubes at  12300 x g, 00:10:00 . Carefully pipette off and discard the liquid phase with as little disruption to the cell pellet as possible. Leave a small amount of liquid volume in the tube if the pellet is too loose.
- 18 Re-weigh the screw-cap tubes containing cell pellets and calculate and record the wet weight by subtracting the empty tube weight.
- 19 For DNA extractions, we aim for a wet pellet weight of  50 mg -  100 mg although we have extracted enough DNA for metagenomic analysis from as low as  25 mg wet pellet weights. If pellets are not used immediately, store in  -80 °C freezer.

10m



Note

For DNA extraction, we use the **Qiagen DNeasy PowerSoil Pro kit** – you can add the lysis buffer to your pellet and transfer the resuspended sample to the bead tube. For the final elution step, we elute in  50 µL of the C6 elution buffer of the kit.



Protocol references

References:

1. Day, JG. (2007). Cryopreservation of microalgae and cyanobacteria. Cryopreservation and freeze-drying protocols, 141-151.
2. Rastoll, MJ, Ouahid, Y, Martín-Gordillo, F, et al. (2013). Journal of applied phycology, 25(5), 1483-1493.