2

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# Cyanobacterial Growth, Harvest, and Genomic DNA Prep V.2

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This protocol is a method for the growth of terrestrial and freshwater cyanobacteria in liquid Z8 medium and the subsequent harvesting and genomic DNA extraction. Life history traits of these microorganisms such as firm cell walls, exopolysaccharide secretions, and variability in growth rates present challenges to studying their genotype. Our approach establishes a generalizable protocol to grow diverse cyanobacteria under the same conditions and a robust DNA extraction technique that produces high-quality low to medium molecule size DNA for Illumina genome sequencing.

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protocol

Ward RD, Stajich JE, Johansen JR, Huntemann M, Clum A, Foster B, Foster B, Roux S, Palaniappan K, Varghese N, Mukherjee S, Reddy TBK, Daum C, Copeland A, Chen IA, Ivanova NN, Kyrpides NC, Shapiro N, Eloe-Fadrosh EA, Pietrasiak N, Metagenome Sequencing to Explore Phylogenomics of Terrestrial Cyanobacteria. Microbiology Resource Announcements 10(22). doi: 10.1128/MRA.00258-21

Cyanobacteria, DNA extraction, MiSeq, Metagenomes

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## Biomass growing conditions 2w

2w

1 ▪ Transfer **100 mL** sterile liquid Z8 media into a Sterile 250mL Polycarbonate Erlenmeyer Flask **VWR Avantor Catalog #89095-270**

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Carmichael, W. W. (1986). Isolation, culture, and toxicity testing of toxic freshwater cyanobacteria (blue-green algae). In Shilov, V. [Ed.] Fundamental research in homogenous catalysis. Volume 3. Gordon & Breach, New York, pp. 1249–62.

- Label the flask with the strain ID.
- Inoculate with cyanobacterial specimen.
- Set vented cap with a 0.22µm pore-size PTFE membrane to the "open" position to allow for gas exchange.
- Secure flask into an orbital shaker at **100 rpm** beneath a fluorescent light at 35-40 µmole·m−2·s−1 and allow to grow until confluent or senescent.




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- Growth period may vary from 2 - 8 weeks depending on growth rate of cyanobacteria species.

## Harvesting and preserving specimens in a biological safety hood 1d

2

- Gently pour cyanobacterial biomass of a particular species into a labelled sterile **50 mL conical tube** (Falcon® Centrifuge Tubes, Polypropylene, Sterile, Corning VWR Cat Nr. 21008-936).

3

- Centrifuge **5000 x g, 00:05:00**
- Decant liquid Z8 media.
- Add **50 mL** liquid Z8 media to tube, cap, and shake vigorously to dislodge potential bacterial contaminants from the cyanobacteria biomass.

4

- Repeat **Step 3** three times

5

- Add a final **50 mL** liquid Z8 media to tube.
- Lightly close cap.
- Wrap with foil and place in refrigerator at **4 °C** for **24 hours** to halt photosynthesis and chromosomal replication.

6

- Carefully decant remaining liquid Z8 media without centrifugation.
- Retrieve sample from tubes with a sterile **inoculation loop** (Globe Scientific Sterile Rigid Inoculating Loops ThermoFisher Cat Nr. 22-170-204) into sterile **1.5 mL Eppendorf tubes** (Fisherbrand™ Locking-Lid Microcentrifuge Tubes with Polypropylene Snap-Cap Cat Nr.: 02-681-284)
- Centrifuge **5000 x g, 00:03:00**
- Remove supernatant Z8 media with a **P1000 pipette** and discard.

7

- Place tubes into liquid nitrogen for **5 minutes.**
- Immediately transfer to **-80 °C** freezer.

## Genomic DNA Prep 1h

8 13m 10s

- Thaw biomass on ice.
- Transfer ca. **500 µL** biomass per **PowerLyzer® bead beating tube** using a sterile **inoculation loop**.



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- Place bead tube and balance in the **homogenizer**.

Precellys
Homogenizer
**Bertin** **P000669-PR240-A**

Process samples during four stages, then allow the **homogenizer** to cool down before turning off.
Apply the following setup:

**5500 rpm, 00:00:45** , pause **00:00:05**
**5500 rpm, 00:00:45** , cool down **00:05:00**
**5500 rpm, 00:00:45** , pause **00:00:05**
**5500 rpm, 00:00:45** , cool down **00:05:00**

9

- Process samples with the Qiagen DNeasy® PowerLyzer® Microbial Kit **Qiagen Catalog #12255-50** , following the manufacturer protocol, with a modified elution step as follows.

- Place spin column into sterile **1.5 mL Eppendorf tube.**

- Transfer **15 µL** PowerLyzer® **elution buffer** to the center of the column membrane.
- Incubate at room temperature for **3 minutes**.
- Centrifuge **1000 x g, 00:03:00**

- Add an additional **25 µL elution buffer** to spin column.
- Incubate at room temperature for 3 minutes.
- Centrifuge **10000 x g, 00:03:00**

10 Store genomic DNA at **-20 °C** until library prep and sequencing.



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