

May 24, 2024

How to extract cyanobacterial genome

 [Microbiology Resource Announcements](#)

DOI

dx.doi.org/10.17504/protocols.io.rm7vzjyn8lx1/v1

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Protocol Citation: Leonardo Ken Okumura 2024. How to extract cyanobacterial genome. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.rm7vzjyn8lx1/v1>

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Protocol status: Working

We use this protocol and it's working



Created: May 02, 2024

Last Modified: May 24, 2024

Protocol Integer ID: 99098

Keywords: cyanobacteria, genome, bacteria, genome sequence, cyanobacterial genome this protocol, cyanobacterial genome, lysozyme, sodium iodide

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Abstract

This protocol describes how to extract cyanobacterial genomes. The main treatments are carried out by sodium iodide, lysozyme, proteinase K and SDS.

Materials

- Extraction Buffer (120 mM NaCl, 50 mM EDTA, pH 8.0)
- Extraction Buffer with Lysozyme (extraction buffer and 10 mg/mL lysozyme)
- Proteinase K solution (20 mg/mL)
- Saturated NaI solution
- 10% SDS solution (w/v)
- PCI (phenol/chloroform/isoamyl alcohol (25:24:1))
- RNaseA solution (20 mg/mL)
- Isopropanol
- 70% ethanol
- Cyanobacteria culture

Troubleshooting



Extracting cyanobacteria genome

- 1 Centrifuge the cyanobacteria culture at 16,000 x g for 10 minutes and remove the supernatant.
- 2 Add 400 μ L of Extraction Buffer and 100 μ L of Saturated NaI Solution to the pellet, and mix well.
- 3 Incubate at 37°C for 30 minutes.
- 4 Centrifuge at 177,000 x g for 5 minutes and remove the supernatant.
- 5 Add 100 μ L of Extraction Buffer to the pellet and mix. Then centrifuge at 177,000 x g for 5 minutes and remove the supernatant.
- 6 Add 500 μ L of Extraction Buffer with Lysozyme to the pellet and mix.
- 7 Incubate at 37°C for 2 hours.
- 8 Add 5 μ L of Proteinase K Solution and 15 μ L of SDS, and mix.
- 9 Incubate at 37°C overnight.
- 10 Add 125 μ L of SDS, and mix.
- 11 Incubate at 37°C for more than 3 hours.
- 12 Add an equal volume of PCI and gently rotate for 5 minutes.



- 13 Centrifuge at 4,000 rpm for 10 minutes at room temperature, and transfer the supernatant to a new tube.
- 14 Add 3 μ L of RNase A, and mix.
- 15 Incubate at 37°C for 30 minutes.
- 16 Repeat steps 12 and 13.
- 17 Add isopropanol in more than equal volume and mix.
- 18 Centrifuge at 16,000 x g for 1 minute at 4°C and remove the supernatant.
- 19 Repeat steps 17 and 18, replacing isopropanol with 70% ethanol.
- 20 Air-dry the pellet and resuspend in distilled water.