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How to extract cyanobacterial genome

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Leonardo Ken Okumura[1]

[1]Tokyo Institute of technology

Leonardo Ken Okumura
Tokyo Institute of technology

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

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 Lisozyme (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

Extracting cyanobacteria genome

1 Centrifuge the cyanobacteria culture at 16,000 x g for 10 minutes and remove the supernatant.

2 Add 400 µL of Extraction Buffer and 100 µL 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 µL 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 µL of Extraction Buffer with Lysozyme to the pellet and mix.

7 Incubate at 37°C for 2 hours.

8 Add 5 µL of Proteinase K Solution and 15 µL of SDS, and mix.

9 Incubate at 37°C overnight.

10 Add 125 µL 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 µL 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.