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Isolation of total DNA from Synechocystis sp. PCC 6803

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1 Works for me dx.doi.org/10.17504/protocols.io.gufbwtn

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ABSTRACT

This protocol can be used for extraction of total genomic DNA from *Synechocystis* sp. PCC 6803.

SAFETY WARNINGS

Wear goggles, a lab coat and gloves when handling chloroform and phenol. Work under the fume hood.
Wear googles when handling liquid nitrogen.

Buffers required:

1 TE-Puffer : 10 mM Tris/HCl ; 1 mM EDTA ; pH 8.0
TE+S-Puffer : 25% (w/v) Sucrose; 50 mM Tris/HCl ; 1 mM EDTA, pH 8.0

Culturing

2 Grow 50 mL Synechocystis culture to the end of the logarithmic phase ($OD_{750} \approx 1.0$).
Centrifuge at 4800 rpm and 4°C for 7 minutes. Remove supernatant.

Wash steps:

3 Resuspend cells in 10 mL TE-buffer, centrifuge at 4800 rpm and 4°C for 7 minutes. Remove supernatant.

4 Repeat washing step. Resuspend pellet in 1 mL TE-buffer. Flash freeze in liquid nitrogen or dry ice.

Cell lysis

5 Add 5 mg · mL^{-1} lysozyme (optional: + 100 mM EDTA).
Incubate 1 hour at 37˚C.
01:00:00

6 Add 14,4 µL Proteinase K (20 mg/mL stock) + 100 µL 20 % SDS.
Incubate 1 hour at 60 ˚C. (Alternatively, incubation can be carried out at 37 ˚C for 16 hours).
01:00:00

DNA extraction:

7 Add 1 volume of Phenol/Chloroform (1:1 v/v). Mix well.

Centrifuge for 10 min at 13000 rpm and 4° C.

8 Transfer upper, aqueous phase to a fresh tube.
Add 1 volume of Phenol/Chloroform (1:1 v/v). Mix well.
Centrifuge for 10 min at 13000 rpm and 4° C.

9 Transfer upper, aqueous phase to a fresh tube.
Add 1 volume of Chloroform in order to remove residual phenol. Mix well.
Centrifuge for 10 minutes at 13000 rpm and 4° C.

DNA precipitation

10 Add 0.7 volumes of isopropanol to the sample. Incubate for 5 minutes at RT.
00:05:00

11 Centrifuge for 30 minutes at 13000 rpm and 4° C.

12 Wash pellet with 1 mL 70 % EtOH.
Centrifuge for 10 minutes at 13000 rpm and 4˚C.

13 Air-dry pellet for 1 hour.
01:00:00

RNase treatment:

14 Resuspend DNA pellet in 30-100 µL TE-buffer.
Add 2 µL RNase A
Incubate 30 minutes at 37˚C.
00:30:00

15 Heat inactivate RNase A by incubating for 10 minutes at 72 ˚C.
00:10:00