genomic DNA-extraction

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ABSTRACT

DNA isolation protocol for quick and rapid extraction of genomic DNA from Chlamydomonas reinhardtii cells. (also works for cyanobacteria)
This protocol is based on a paper published by Newman et al 1990 (PMID: 1981764).
The protocol is designed to be used for single colonies or 1ml of cell culture but can be used for a larger amount of biomass if scaled correctly. Here I describe the protocol for 10ml of overnight culture.

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MATERIALS TEXT

TEN buffer (10mM Tris-HCl, 10mM EDTA [Ethylenediaminetetraacetic acid], 150mM NaCl); SDS-EB (2% SDS, 400mM NaCl, 40mM EDTA, 100mM Tris-HCl, pH8.0), Nuclease free water, phenol/Chloroform:isoamyl alcohol(1:1), Chloroform:isoamyl alcohol, 70% Ethanol, 100% Ethanol, TE Buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0)

Buffer preparation

1
- TEN Buffer (10 mM Tris-HCl, 10 mM EDTA, 150 mM NaCl)
- SDS-EB Buffer (2% SDS, 400 mM NaCl, 40 mM EDTA, 100 mM Tris-HCl, pH 8.0)
- TE Buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0) [autoclaved]

Resuspension and lysis

2 Pellet the 10ml overnight culture of your algae or cyanobacteria in a 15ml falcon at 5.000g.

3 Resuspend in 5ml of TEN Buffer by vortexing, spin for 10 sec. and aspirate off supernatant.

4 Resuspend cells in 150 ul H2O on ice and add 300 ul of SDS-EB buffer, vortex to mix.

Phenol Chloroform washing

5 Extract once with 3.5 ml phenol/Chloroform:isoamyl alcohol(1:1) for a few min by vortexing, separate phases by centrifugation for 5 min., transfer aq. phase to a new falcon tube.

6 Extract once with 3 ml Chloroform:isoamyl alcohol (24:1), transfer aq. to a new tube.

7 Repeat step in order to maximize purity.

Precipitation and Resuspension

8 Add 2 volumes abs. ethanol, incubate on ice for 30 min., centrifuge for 10 min., wash pellet once with 200 ul 70% ethanol.

9 Dry at 60°C until all ethanol evaporated and resuspend in desired volume of TE Buffer.

10 Incubate at 50 °C for 1 to 2 h to ensure complete re-suspension. (Can be extended to overnight incubation)

11 Measure the quality and quantity of your DNA using a NanoDrop.

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