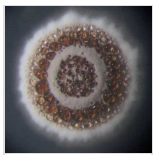

Version 2

Feb 19, 2021

DNA extraction and quantification V.2

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2 Works for me dx.doi.org/10.17504/protocols.io.bsj4ncqw

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ABSTRACT

DNA extraction and quantification

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MATERIALS TEXT

Materials

Polypropylene tubes of 1,5 µL
Autoclavable glass flasks 200 mL
Petri dishes with grown bacterial cells
Disposable inoculating loops
Absorbent paper
Tips of 10 µL, 200 µL, 1000 µL
Permanent marker for labeling
Gloves

Reagents

λ phage (50 ng/µL)
Trizma base
EDTA
HCl
NaCl
Lysozyme (100 mg/mL)
Proteinase K (20 mg/mL)
Sodium dodecyl sulfate (SDS)
Cetyltrimethylammonium bromide (CTAB)
Chloroform
Isoamyl alcohol
Isopropyl alcohol
Ethanol
MilliQ water

Solutions

TE 1X (10mM of Tris-HCl, 1mM of EDTA pH 8,0)
TBE 1X (89mM Tris-borate, 89mM boric acid, 2mM EDTA) *revisar
Sodium dodecyl sulfate 10 % (SDS)
Chloroform/isoamyl alcohol (24:1)
Cetyltrimethylammonium bromide/NaCl (0,03 M/0,07 M)
NaCl 5M
Ethanol 70 %
Ethanol 100 %

Other

Micropipette of 10 µL, 200 µL, 1000 µL
Speedvac
Centrifuge
Vacuum manifold and pump
Analytical balance
Micropipette 10 µL, 200 µL, 1000 µL
Freezer

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1 Resuspend previously grown bacterial cells (approximately 50 mg of bacterial fresh biomass) in 400 µL de TE 1X (10mM of Tris-HCl, 1mM of EDTA pH 8,0).

2 Add 1 µL of lysozyme (100mg/ml) shortly vórtex, and incubate for 1 h at 37 °C.

3 Add 67,5 µL of SDS 10 % (pre-heated at 65 °C) and 2,5 µL of Proteinase K (10 mg/mL) and shortly vórtex, and incubate at 65 °C for 10 min.

4 Add 100 µL of NaCl at 5M and 100 µL of a solution of Cetyltrimethylammonium Bromide (CTAB)/NaCl (0,03 M/0,07 M), both pre-heated at 65 °C, and vortex until observing a whitish color and incubate at 65 °C for 10 min.

5 Add 750 µL of chloroform/isoamyl alcohol (24:1), vortex for 10 s and centrifugate at 12.000 rpm for 5 min.

6 Transfer the supernatant (approximately 800 µL) to a new tube and add 0,6 volumes of isopropyl alcohol to precipitate the DNA and gently invert the tube.

6.1 Store at -20 °C for 30-60 min in case precipitated DNA is not observed.

7 Centrifugate at 4 °C at 12000 rpm for 5 min.

8 Discard the supernatant and add 1 mL of ice-cold ethanol 70% (-20 °C).

9 Centrifugate at 12.000 rpm for 5 min and discard the supernatant.

10 Add 1 mL of ice-cold ethanol 100 % (-20 °C).

11 Centrifugate at 12.000 rpm for 5 min and discard the supernatant.

12 Centrifugate at 12.000 rpm at 4 °C for 1 min to let the ethanol evaporate at room temperature.

Invert the tubes, place them on absorbent paper, and then use a speedvac at 36 °C for 30 min to let them completely

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13 dry.

14 Resuspend the pellet in a volume of 20 µL of MilliQ water (add more water if necessary, according to the concentration of the pellet).

15 Store the DNA at -20 °C.

16 Check the DNA quality by migrating different concentrations of it and using a control λ phage concentration of 50 ng/µL in an agarose gel with 1 % of TBE 1X ran for 30 min at 5 V per cm^2

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