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Extraction of gDNA from Synechocystis 6803

Dennis Dienst[1]

[1]Department of Chemistry - Ångström, Uppsala

1 Works for me dx.doi.org/10.17504/protocols.io.k99cz96

CyanoWorld

Dennis DD. Dienst
Department of Chemistry - Ångström, Uppsala

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

null

MATERIALS

NAME	CATALOG #	VENDOR
RNase A 10mg/ml, DNase and Protease-free	EN0531	Thermo Scientific
Phenol/Chloroform/Isoamyl alcohol (25:24:1)	A156	Carl Roth
2-propanol	6752	Carl Roth

Preparation of buffer solutions

1 **TE buffer**:
10 mM Tris-HCl
1 mM EDTA ; pH 8.0

TES buffer:
50 mM Tris-HCl
1 mM EDTA, pH 8.0
25% (w/v) Saccharose

Cell harvest & disruption

2 - Centrifuge 50 mL liquid culture (OD ~ 0.4-1.0) for 7 min at 4000 g and 4 °C
00:07:00 **4000 g**

3 - Resuspend pellet in 10 mL TE buffer
- repeat centrifugation (step 1)

go to step #2

4 - Repeat wash (step2)

go to step #3

5
- Resuspend pellet in 1 mL TES buffer
- freeze in liquid nitrogen

wear goggles when working with liquid nitrogen
https://en.wikipedia.org/wiki/Liquid_nitrogen

6
- add 5 mg × mL $^{-1}$ (spatula tip) lysozyme, 3.2 units proteinase K and 100 µL 20% SDS
- incubate for 1h at 37 °C

01:00:00

37 °C

7
- incubate for 1h at 60°C under gentle agitation
- alternatively: 16h (o/n) at 37 °C

01:00:00

60 °C

Phenol-chloroform extraction

8
- add 1 Vol.
- add 1 Vol. Phenol/Chloroform/Isoamyl alcohol (25:24:1) and mix
- centrifuge for 10 min at 12000 g and 4°C

00:10:00 **12000 g**

4 °C

9
- transfer upper (aqueous) phase to fresh 1.5 mL tube
- add 1 Vol. Phenol/Chloroform/Isoamyl alcohol (25:24:1) and mix
- centrifuge for 10 min at 12000 g and 4°C

00:10:00 **12000 g**

4 °C

10
- Repeat step 8

go to step #9

DNA precipitation

11
- transfer upper (aqueous) phase to fresh 1.5 mL tube
- add 0.7 Vol. 2-propanol (isopropanol)
- incubate for 5 min at room temperature

00:05:00 **room temperature**

12
- centrifuge for 30 min at 12000 g and 4 °C

00:30:00 **12000 g**

4 °C

13
- carefully remove 2-propanol
- add 300 µL 70% EtOH
- air-dry pellet for 1h at room temperature

(i.e. leave tube open on your bench and enjoy a coffee or two)

DNA pellet might be hardly visible

01:00:00 room temperature

14
- Resuspend DNA in 30-100 µL TE buffer

optional: RNA removal

15
- add 2µL RNase A
- incubate 30 min at 37 °C
- incubate 10 min at 72 °C

16 Store DNA at -20 °C