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Phenol/chloroform extraction of DNA from cyanobacteria V.2

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Robbie Martin[1], Steven W. Wilhelm[1]

[1]University of Tennessee, Knoxville

The Aquatic Microbial E...

Robbie Martin

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We use this protocol and it's working

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Abstract

Phenol/chloroform-based DNA extraction from cells pre-treated with RNase A, lysozyme, proteinase K, and SDS. The protocol was optimized for extracting DNA from *Microcystis aeruginosa*, but works well for other cyanobacteria.

Materials

MATERIALS

- Sodium acetate **P212121**
- RNase A **Qiagen Catalog #**19101
- Chloroform/IAA **Amresco Catalog #**X205
- TE buffer
- Phenol
- 70% Ethanol
- Proteinase K **Thermo Fisher Scientific Catalog #**EO0491
- Sodium Dodecyl Sulfate, 100gm **Promega Catalog #**H5113
- Lysozyme **Bio Basic Inc. Catalog #**LDB0308.SIZE.1g
- 100% Ethanol

Safety warnings

See **MSDS** for safety and warnings.

Pre-treatment of cells with RNase A, lysozyme, proteinase K, and SDS

1 Concentrate ~4 mL of cellular culture in an Eppendorf tube by centrifugation at 10,000 xg for ~5 min.

4 µL cellular culture

00:05:00 centrifugation at 10,000 xg

2 Discard supernatant.

3 Resuspend pellet in 425 µL of standard TE buffer.

425 µL TE buffer

Note

Standard TE buffer

-10 mM Tris
-1 mM EDTA
-pH 8

4 Add 50 µL of 100 µg/mL RNase A TER buffer (RNase A plus TE buffer) for a final concentration of 10 µg/mL RNase A.

50 µL RNase A TER buffer

5 Add 25 µL of 100 mg/mL lysozyme for a final concentration of 5 mg/mL lysozyme.

25 µL of 100 mg/mL lysozyme

6 Incubate at 37 °C for 20 minutes.

37 °C incubation

00:20:00 incubation

7 Add 50 µL of 1 mg/mL proteinase K for a final concentration of 100 µg/mL proteinase K.

8 Add 50 µL of 10% sodium dodecyl sulfate.

50 µL 10% sodium dodecyl sulfate

9 Incubate at 50 °C for 2 hr.

50 °C incubation

02:00:00 incubation

Phenol/chloroform extraction

10 Add 250 mL phenol (pH 8.0). Mix gently and completely.

250 µL phenol (pH 8.0)

11 Add 250 mL chloroform/isoamyl alcohol (24:1), mix gently and completely.

250 µL chloroform/isoamyl alcohol (24:1)

12 Spin at maximum speed in benchtop centrifuge for at least 2 minutes.

Note

Longer spin times, (up to 10 min.) may help in separating the phases.

13 Transfer aqueous (top) layer to clean Eppendorf tube without removing any of the organic layer.

14 Remove the last of the aqueous layer along with some of the organic layer (to ensure all aqueous volume is collected) and add it to a new, clean Eppendorf tube for later re-extraction.

Note

This re-extraction will significantly increase DNA recovery.

15
- Repeat steps 10, 11 and 12 twice (a total of 3 phenol extractions) or until no visible protein layer is seen.
- Each time, remove the last of the aqueous layer along with some of the organic layer and add to the 're-extraction' Eppendorf tube (refer to step 14).

go to step #10 At least 3 phenol extractions, or until no visible protein layer is seen.

Note

Protein layer will be white scum at interface.

go to step #14 Add to 're-extraction' Eppendorf tube.

16 Add 500 µL of chloroform and mix gently.(1/2)

500 µL chloroform

17 Spin at maximum speed in benchtop centrifuge for at least 2 minutes. (1/2)

> Note
>
> This will help remove traces of phenol.

00:02:00 centrifugation

18 Transfer aqueous (top) layer to clean Eppendorf tube without removing any of the organic layer.(1/2)

19 Repeat steps 16-18 to remove all phenol. (2/2)

go to step #16 Repeat phenol removal

20 Extract the mixed aqueous/organic contents of the 're-extraction' Eppendorf tube and follow extraction steps 10-19. Combine the final aqueous layer with that collected above.

go to step #10 extraction steps for aqueous/organic contents

Ethanol precipitation

21 Add 0.1 volume of 3 M sodium acetate to the collected aqueous phase and mix gently.

22 Add 2 volumes of ice cold 100% ethanol and gently mix well.

> Note
>
> Strings of precipitating DNA should become visible.

23 Place tube in -80 °C freezer until ethanol mixture is partially frozen (~1 hr.)

-80 °C freezer

01:00:00 approximate time for partial ethanol freeze

24 Spin at maximum speed in benchtop centrifuge at 4 °C for 30 min.

00:30:00 centrifugation

4 °C centrifugation

25 Discard supernatant and very carefully aspirate the remaining droplets of liquid from the tube without disrupting the DNA pellet.

26 Wash the DNA pellet by adding 500 µL of ice-cold 70% ethanol to the tube and pipetting it gently, several times.

500 µL ice-cold 70% ethanol

27 Spin at maximum speed in a benchtop centrifuge at 4 °C for 15 min.

00:15:00 centrifugation

4 °C centrifugation

28 Discard supernatant and carefully aspirate the remaining droplets of liquid from the tube without disrupting the DNA pellet.

29 Place in a heater block at ~37 °C for less than 5 minutes to evaporate all ethanol.

Note

Be careful! DO NOT dry to completion! Alternatively, you can air dry to remove the ethanol.

00:04:59 ethanol drying

37 °C heater block

30 Re-suspend the DNA pellet in 50-100 µL of TE buffer or water (depending on downstream use and needed concentration).

50 µL TE buffer or water

31 Freeze at -20 or -80 °C.