Phenol/chloroform extraction of DNA from cyanobacteria

Version 2

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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.

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Materials

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

Protocol

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

Step 1.

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

AMOUNT
4 ml Additional info: cellular culture

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

Step 2.

Discard supernatant.

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

Step 3.

Resuspend pellet in 425 µL of standard TE buffer.

AMOUNT
425 µl Additional info: TE buffer

NOTES
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Standard TE buffer

-10 mM Tris

-1 mM EDTA

-pH 8

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

Step 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.

AMOUNT
50 µl Additional info: RNase A TER buffer

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

Step 5.

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

AMOUNT
25 µl Additional info: of 100 mg/mL lysozyme

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

Step 6.

Incubate at 37 °C for 20 minutes.

TEMPERATURE
37 °C Additional info: incubation

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

Step 7.

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

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

Step 8.

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Add 50 µL of 10% sodium dodecyl sulfate.

AMOUNT
50 µl Additional info: 10% sodium dodecyl sulfate

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

Step 9.

Incubate at 50 °C for 2 hr.

TEMPERATURE
50 °C Additional info: incubation

Phenol/chloroform extraction

Step 10.

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

AMOUNT
250 ml Additional info: phenol (pH 8.0)

Phenol/chloroform extraction

Step 11.

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

AMOUNT
250 ml Additional info: chloroform/isoamyl alcohol (24:1)

Phenol/chloroform extraction

Step 12.

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

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

Phenol/chloroform extraction

Step 13.

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

Phenol/chloroform extraction

Step 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.

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This re-extraction will significantly increase DNA recovery.

Phenol/chloroform extraction

Step 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).

GOTO

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

NOTES

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Protein layer will be white scum at interface.

Phenol/chloroform extraction

Step 16.

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

AMOUNT

500 µl Additional info: chloroform

Phenol/chloroform extraction

Step 17.

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

NOTES

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This will help remove traces of phenol.

Phenol/chloroform extraction

Step 18.

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

Phenol/chloroform extraction

Step 19.

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

GOTO

Repeat phenol removal -> go to step #16

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Phenol/chloroform extraction

Step 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.

GOTO

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

Ethanol precipitation

Step 21.

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

Ethanol precipitation

Step 22.

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

NOTES

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Strings of precipitating DNA should become visible.

Ethanol precipitation

Step 23.

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

TEMPERATURE

-80 °C Additional info: freezer

Ethanol precipitation

Step 24.

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

TEMPERATURE

4 °C Additional info: centrifugation

Ethanol precipitation

Step 25.

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

Ethanol precipitation

Step 26.

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

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AMOUNT

500 µl Additional info: ice-cold 70% ethanol

Ethanol precipitation

Step 27.

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

TEMPERATURE

4 °C Additional info: centrifugation

Ethanol precipitation

Step 28.

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

Ethanol precipitation

Step 29.

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

TEMPERATURE

37 °C Additional info: heater block

NOTES

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

Ethanol precipitation

Step 30.

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

AMOUNT

50 µl Additional info: TE buffer or water

Ethanol precipitation

Step 31.

Freeze at -20 or -80 °C.

Warnings

See MSDS for safety and warnings.

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