Phenol/chloroform extraction of DNA from cyanobacteria

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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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Protocol

Pre-treatment of cells with RNase A, lysozyme, proteinase K, and SDS
Step 1.

Concentrate 4 mL of cell culture in an eppendorf tube by centrifuging at 10,000 xg for 5 min. Discard supernatant.

Pre-treatment of cells with RNase A, lysozyme, proteinase K, and SDS
Step 2.

Re-suspend pellet in 425 µL of standard TE buffer (10 mM Tris, 1 mM EDTA, pH 8).

Pre-treatment of cells with RNase A, lysozyme, proteinase K, and SDS
Step 3.

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.

Pre-treatment of cells with RNase A, lysozyme, proteinase K, and SDS
Step 4.

Add 25 µL of 100 mg/mL lysozyme for a final concentration of 5 mg/mL lysozyme. Incubate at 37 °C for 20 min.

Pre-treatment of cells with RNase A, lysozyme, proteinase K, and SDS
Step 5.

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 6.

Add 50 µL of 10% sodium dodecyl sulfate. Incubate at 50 °C for 2 hr.

Phenol/chloroform extraction

Step 7.

Add 250 mL phenol (pH 8.0), gently but thoroughly mix.

Phenol/chloroform extraction

Step 8.

Add 250 mL chloroform/isoamyl alcohol (24:1), gently but thoroughly mix. Spin at maximum in a benchtop centrifuge for at least 2 minutes. Longer spin times (up to 10 min.) may help in separating the phases.

Phenol/chloroform extraction

Step 9.

Transfer aqueous (top) layer to clean eppendorf tube without removing any of the organic layer. Remove the last of the aqueous layer along with some of the organic layer (to ensure all aqueous volume is collected) to a second clean tube for later re-extraction. This re-extraction will significantly increase DNA recovery.

Phenol/chloroform extraction

Step 10.

Repeat steps 7 and 8 twice (a total of 3 phenol extractions) or until no visible protein layer is seen. Protein layer will be white scum at interface. Each time, remove the last of the aqueous layer along with some of the organic layer to the 're-extraction' eppendorf tube (refer to step 9).

Phenol/chloroform extraction

Step 11.

Add 500 µL of chloroform and gently mix. Spin at maximum in a benchtop centrifuge for at least 2 minutes. (This will help remove traces of phenol.)

Phenol/chloroform extraction

Step 12.

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

Phenol/chloroform extraction

Step 13.

Repeat steps 11 and 12 to remove all phenol.

Phenol/chloroform extraction

Step 14.

Extract the mixed aqueous/organic contents of the "re-extraction" eppendorf tube following steps 7-13. Combine the final aqueous layer with that collected above.

Ethanol precipitation

Step 15.

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

Ethanol precipitation

Step 16.

Add 2 volumes of ice cold 100% ethanol and gently mix well. Strings of precipitating DNA should become visible.

Ethanol precipitation

Step 17.

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

Ethanol precipitation

Step 18.

Spin at maximum in a benchtop centrifuge at 4 °C for 30 min. Discard supernatant and very carefully aspirate the remaining droplets of liquid from the tube without disrupting the DNA pellet.

Ethanol precipitation

Step 19.

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

Ethanol precipitation

Step 20.

Spin at maximum in a benchtop centrifuge at 4 °C for 15 min. Discard supernatant and carefully aspirate the remaining droplets of liquid from the tube without disrupting the DNA pellet.

Ethanol precipitation

Step 21.

Place in a heater block at 37 °C for less than 5 minutes to evaporate all ethanol. Be careful! DO NOT dry to completion! Alternatively, you can air dry to remove the ethanol.

Ethanol precipitation

Step 22.

Re-suspend the DNA pellet in 50-100 µL of TE buffer or water (depending on downstream use and needed concentration). Freeze at -20 or -80 °C.