DNA Extraction from Environment Samples – Grind plus kit Method

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This is the most recommended protocol till now. Our classic cell disruption method (freeze grind and SDS lysis in the classic extraction buffer) can provide good DNA recovery from various microorganisms with perfect intactness. Mobio kits, which are easier and faster than gel purification, have excellent performance in removing PCR inhibitors to achieve high DNA purity.

We are developing a new protocol which is more efficient and provides even higher DNA yield. However, the new protocol results in obvious DNA shearing, thus cannot replace this protocol if DNA intactness really matter for your study.

Solutions:

Extraction buffer

6.8 mL 1 M NaH_2PO_4 (*monobasic*)

93.2 mL 1 M Na_2HPO_4 (*dibasic*)

Combine phosphate sol., pH to 8.0 with NaOH, continue with remaining ingredients

200 mL 0.5 M EDTA, pH 8.0

100 mL 1 M Tris-HCl, pH 8.0

300 mL 5 M NaCl

100 mL 10% CTAB [*for **filter samples**, leave CTAB out]*

Bring to 1 L with DI water, **if CTAB is left out; bring to 900 mL with DI water**

(Final concentrations: 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4, 0.1 M EDTA, 0.1 M Tris-HCl, 1.5 M NaCl, 1% CTAB)

Other Chemicals Needed:

Proteinase K

10 mg mL^{-1} (*store at -20C*)

20 % SDS (*pre-made*)

70 % Ethanol (*cold, store at -20C*)

2-Isopropanol

0.5 M EDTA, pH 8.0

1 M Tris-HCl

* 100x TE (Sigma, Catalog# T9285-100ML)

*(optional) Chloroform:isoamyl alcohol (24:1)

Combine the chloroform and isoamyl alcohol and store in a dark or foil covered bottle and keep it in the flammables cabinet below the fume hood.

Kit

MO BIO PowerClean® Pro DNA Clean-Up Kit (MO BIO, 12997-50).
or MO BIO PowerSoil® DNA Isolation Kit (MO BIO, 12888-100 or 12888-50).

Other Notes:

The Oak Ridge tubes used in this protocol should not be autoclaved. If the tubes are autoclaved, the DNA pellet does not form a tight pellet and can be difficult to see. To clean the tubes, rinse them in DI water after use and then boil for 20 min in DI water. Once cooled, rinse the tubes with 70% EtOH and dry.

Steps:

(1) Weigh out 1 g of sample into a sterile mortar (use the smaller or medium mortar in our lab). *Only take out one sample at a time to minimize DNA degradation from being at room temperature.*
Add 0.5 g of sterile sand to the mortar. *More sand can be added if needed.* Add liquid N_2 to the mortar to cool the sand and mortar. *Be generous with the N_2.*
Note: If 1 g soil is not enough, you can use more soil and increase the volumes of solutions (i.e. extraction buffer, proteinase K, SDS and isopropanol) proportionally in following steps. This protocol is also applicable for other types of environment samples (sludge, water, filter, wood, etc.), but the amount of sample depends.

(2) Add more N_2 to cover the sand/sample and begin grinding once the N_2 has evaporated. Try to contain sample to a small area of the mortar. Grind until the sample starts to thaw. If possible, keep the sample frozen while grinding (this can be easy if the soil is dry and sandy).
Note: If the sample is difficult to grind when frozen, you can allow it to thaw, but make sure to add 0.2~0.4 mL of extraction buffer to the sample. The buffer with inhibit degradation of the DNA when it warms.

(3) Repeat freezing and grinding twice (3 times total).

(4) While the sample is still frozen scrape the sides of the mortar with a spatula to collect the sample in the center of the mortar (add more N_2 if necessary to keep the sample frozen). Transfer this to a fresh 15-mL tube using a spatula.
Note: At this point the sample can be kept frozen (-80 °C) until ready to proceed with DNA extraction

(5) Remove samples from freezer. Add 3.3 mL Extraction Buffer (with CTAB). *The total volume of buffer should be 3.3 mL, so if you added buffer during the freeze-grinding, remember to subtract that amount from the added at this step.*
Note: If your soil is too dirty but contains enough DNA, you may add 5 mL extraction buffer.

(6) Add 12.2 µL proteinase K (10 mg mL^{-1}), mix gently.
Note: If you add 5 mL buffer at step (5), add 18.5 µL proteinase K.

(7) Incubate at 37 °C for 30 min (*keep in a 37 °C water bath and invert every 5-10 min*)

(8) Add 0.37 mL 20 % SDS, mix gently
Note: If you add 5 mL buffer at step (5), add 0.56 mL 20% SDS.

(9) Incubate at 65 °C for 2 h with gentle inversion every 15-30 min.

(10) Centrifuge 20 min, 6000 x *g* at 25 °C.

(11) Transfer liquid to an oak ridge tube (*Use the translucent oak ridge tubes*). Try your best to avoid the white surface layer.
Note: If your sample had too much organic contaminant (e.g. protein), you might transfer liquid to a 15-mL conical centrifuge tube (for chloroform extraction).

(12) Add 1.2 mL extraction buffer containing CTAB to the remaining sand pellet and mix.
Note: If you add 5 mL buffer at step (5), add 1.8 mL extraction buffer here.

(13) Add 0.13 mL 20 % SDS, mix gently.
Note: If you add 5 mL buffer at step (5), add 0.2 mL 20% SDS here.

(14) Incubate at 65 °C for 15 min.

(15) Centrifuge 20 min, 6000 x *g* at 25 °C.

(16) Collect supernatant and combine with previous supernatant, avoiding the white surface layer.
Note: If your sample had too much organic contaminant (e.g. protein), extract supernatant with an equal volume of isoamyl:chloroform (1 part isoamyl alcohol, 24 parts chloroform) for 5-10 min by continuous inversion before next step (the rotator located in the fume hood can be used to continuously mix the samples). Centrifuge at 3700 x g, 20 min – use the benchtop centrifuge (the orange or purple capped conical tubes can withstand this speed). Collect the supernatant into a fresh conical tube. Repeat this chloroform extraction once, and then transfer the supernatant into an oak ridge tube.

(17) Add 0.6 volume of 2-isopropanol (*very important that exactly 0.6 volume is added).*

(18) Incubate at -20 or -80 °C overnight. *The cold will help the DNA to precipitate.*

(19) Remove the tube from the freezer and warm in a 37 °C water bath. Make sure the sample is warm and all precipitates have dissolved before proceeding. *Warming the sample prior to centrifugation will dissolve any mineral precipitates that may have formed overnight.*

(20) Centrifuge 15,000 x *g* (RCF) for 20 min at 25 °C *(Make sure the centrifuge is at RT*

– *if it is too cold, mineral precipitates in the sample will be allowed to form)*. Immediately after centrifugation, transfer the supernatant to a fresh tube (*keep the supernatant until you know whether DNA is present*).

(21) Wash the pellet with 1 mL ice-cold 70 % ethanol. Transfer DNA and ethanol to a 1.5-mL tube. If no pellet is visible or the pellet scatters, centrifuge 15,000 x *g*, 5 min. Discard the ethanol.
Note: If the pellet was too dirty, you can wash twice. You may use pipette to remove ethanol as completely as possible.

If you will use PowerClean Pro kit:

(22) Add 100 µl 1x TE (10 mM Tris-HCl, 1 mM EDTA.Na2, pH=8, sterile, DNase free) to dissolved DNA. Mix to make sure DNA is dissolved well.
Note: It is very important to dissolve the DNA well. Pipette is more effective than vortex to make the DNA pellet dissolved. Incubate at 50 ℃ for 2~5 min if the pellet is hard to dissolve.
In some cases, it is better to dilute the crude DNA to 1/2 ~ 1/10 and just use 100 µl diluted DNA for next steps, so that the concentration of humic substance will not exceed the capability of the kit.

(23) Following the manufacture protocol of PowerClean Pro kit to purify DNA. However, use 1x TE (pH=8.0) or water (DNase free) instead of solution DC5 in the kit.
Note: protocol: http://www.mobio.com/images/custom/file/protocol/12997-50.pdf DC5 has no EDTA, so it is good if the further usage of the DNA is sensitive to EDTA. 1x TE is better for long-term storage. However, if you want to get accurate 260/230 value by nanodrop, please use water to elute DNA from spin filter. After nanodrop test, you may add 1/100(v/v) 100x TE to get 1x TE in DNA sample for long-term storage.

(24) Check DNA purity with Nanodrop.
260/280 ~ 1.8, 260/230≥1.7.
Note: If 260/230 is bad, do desalting according to "Community DNA Preparation through hybridization" at http://ieg.ou.edu/protocol.htm (page 10, Desalting protocol). If the DNA concentration is too low, it will be hard to precipitate DNA by acidification and cold ethanol. You may concentrate the DNA to about 100 ng/µl, precipitate DNA and wash the pellet with 10~20 volumes 70% cold ethanol.
If neither 260/230 nor 260/280 is good enough, try to repeat clean-up by PowerClean Pro kit.

(25) Determine the dsDNA concentration by Picogreen test ("dsDNA quantification with PicoGreen" at http://ieg.ou.edu/protocol.htm). Check DNA shearing with agrose gel (0.8% agrose, 105 V, 30 min).

If you will use PowerSoil kit:

(22) Add 430 µl bead solution (MOBIO PowerSoil kit, in the bead tube) to dissolved DNA. Mix to make sure DNA is dissolved well.
Note: It is very important to dissolve the crude DNA well. Pipette is more effective than vortex to make the DNA pellet dissolved. Incubate at 50 ℃ for 2~5 min if the pellet is hard to dissolve.
In some cases, we found it is better to dilute the crude DNA to 1/2 ~ 1/10 and just use 100 µl diluted DNA for next steps, so that the concentration of humic substance will not exceed the capability of the kit.
Materials for next Steps are from MOBIO PowerSoil kit.

(23) Add 250 µl solution C2, vortex 5 s to mix, incubate at 4°C for 5 min.

(24) Centrifuge at 10,000g for 1 min. Transfer 650 µl supernatant to a clean tube.

(25) Add 200 µl solution C3, vortex 5 s to mix, incubate at 4°C for 5 min.

(26) Centrifuge at 10,000g for 1 min. Transfer 700~750 µl supernatant to a 2 ml tube.
Note: If the solution appears very dirty even after treated by C3 (e.g., in brown color), you can repeat the steps (25) and (26) using another 200 µl solution C3 to improve DNA purity. ***However, in some cases, we did find DNA can be largely lost if repeat using C3.***

(27) Add 1.2 ml solution C4, vortex 5 s to mix.
Note: Remember to shake C4 before open it.

(28) Load 675 µl of the sample to the Spin filter (the column). Centrifuge at 10,000 g for 1 min. Discard the flow through. Repeat twice using the same Spin filter for a total of 3 times.

(29) Add 500 µl solution C5 to the Spin filter and centrifuge at 10,000g for 30 s. Discard the flow through. Repeat if your sample is too dirty.
Note: Generally, it is not necessary to wash more than 2 times with C5. The 260/230 ratio may not be better.

(30) Centrifuge Spin filter one more time at 10,000g for 1 min. Carefully transfer the Spin filter to a new 2 ml tube.
Note: If you find some solution C5 remain on the rim, you can use a clean kimwipes to blot it.

(31) Add 100 µl nuclease free water to the center of the filter. Centrifuge at 10,000g for 30 s. Discard the Spin filter.
Note: C6 has no EDTA, so it is good if the further usage of the DNA is sensitive to EDTA. 1x TE (pH=8) is better for long-term storage. However, if you want to get accurate 260/230 value by nanodrop, please use water to elute DNA from spin filter.

After nanodrop test, you may add 1/100(v/v) 100x TE (pH=8) to get 1x TE in DNA sample for long-term storage.

(32) Check DNA purity with Nanodrop.
260/280 ~ 1.8, 260/230≥1.7.
Note: If 260/230 is bad, do desalting according to "Community DNA Preparation through hybridization" at http://ieg.ou.edu/protocol.htm (page 10, Desalting protocol). If the DNA concentration is too low, it will be hard to precipitate DNA by acidification and cold ethanol. You may concentrate the DNA to about 100 ng/μl, precipitate DNA and wash the pellet with 10~20 volumes 70% cold ethanol.
If neither 260/230 nor 260/280 is good enough, try to repeat from step (22) to (32) and use 330 μl when repeat step (22).

(33) Determine the dsDNA concentration by Picogreen test ("dsDNA quantification with PicoGreen" at http://ieg.ou.edu/protocol.htm). Check DNA shearing with agrose gel (0.8% agrose, 105 V, 30 min).

Please feel free to contact Daliang (ningdaliang@ou.edu) for any question or suggestion.