DNA Extraction from Radioative Samples – Grind plus kit Method

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To extract DNA from radioactive sediment samples with low biomass, we are currently not allowed to use chloroform or phenol, and need to use a relatively large amount of sample to get enough DNA. Freeze grind combined with DNeasy PowerSoil kit from QIAGEN for purification could be the best choice.

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.
(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*) or 10% SDS (prepared, commercial)

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)

DNase free water

Kit

DNeasy PowerSoil Kit (QIAGEN Cat No./ID: 12888-100 or 12888-50, previous 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, firstly, to remove radioactive substance remained after last use with paper

towel, then rinse them in DI water and then boil for 20 min in DI water. Once cooled, rinse the tubes with 70% EtOH and dry.

A. Raw DNA extraction

(1) Preparation:
- Radiation protection stuffs.
- 37°C and 65°C water bath.
- Sterile buffer, mortars, pestles, sand, spatulas, tubes, tips.
- Paper towel, 70% ethanol for surface sterile.
- Centrifuge (and 50-ml rotor with conical base) set at room temperature.

(2) Weigh out 10 g of sample into a sterile mortar (use the medium mortar in our lab). *Only take out one sample at a time to minimize DNA degradation from being at room temperature.*
Add 2 g of sterile sands to the mortar. Add liquid N_2 to the mortar to cool the sand and mortar. *Be generous with the N_2.*

(3) 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).*

(4) Repeat freezing and grinding three times (4 times total).
Note: In most cases, you may just smash rocks and frozen chunks into pieces at the first grinding.

(5) 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 50-mL tube using a spatula. Store the tube with ground sample immediately to a -80 °*C* freezer.
Note: At this point the sample can be kept frozen (-80 °C) until ready to proceed with DNA extraction.

(6) Remove samples from freezer. Add 16.5 mL Extraction Buffer (with CTAB) to each 50-ml tube.

(7) Add 61 μL proteinase K (10 mg m^{L-1}), mix gently.

(8) Incubate at 37 °C for 40 min (*keep in a 37 °C water bath and invert every 5-10 min*)
Note: 10 min longer than other protocol due to the cold sample.

(9) Add 3.7 mL 10% SDS (or 1.83 mL 20 % SDS), mix gently

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

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

(12) Transfer liquid to an oak ridge tube (*Use the translucent oak ridge tubes*). Try your best to avoid the white surface layer.

(13) Add 6 mL extraction buffer to the remaining sand pellet and mix.

(14) Add 1.35 mL 10% SDS (or 0.67 mL 20 % SDS), mix gently.

(15) Incubate at 65 °C for 15 min.

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

(17) Collect supernatant and combine with previous supernatant, avoiding the white surface layer.
Note: Try your best to avoid the white surface layer. It is important.

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

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

(20) Preparation:
- 37°C and 50°C water bath.
- Sterile tubes, tips.
- 70 % ice-cold ethanol.
- Centrifuge (and 50-ml rotor with round base) set at room temperature.

(21) Remove the tube from the freezer and warm in a 37 °C water bath. Make sure the sample is warm and all precipitated salt have dissolved before proceeding. *Warming the sample prior to centrifugation will dissolve any mineral precipitates that may have formed overnight.*
Note: It may need 30 min (sometimes even 45 min) to dissolve mineral precipitates as thoroughly as possible.

(22) 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*).

(23) Wash the pellet with 1 mL ice-cold 70 % ethanol. If the pellet goes to pieces, centrifuge at 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. Remember that some pure DNA can be invisible, in the transparent part spreading from the edge of the main pellet. You might lose a part of DNA if you discarded ethanol without centrifuge or transfer before

completely dissolving DNA by buffer in next step.

B. DNA purification using DNeasy PowerSoil kit:

(1) Add 430 µl bead solution (MOBIO PowerSoil kit, in the bead tube) to dissolve 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.
If the sample has a lot of humic substance and enough DNA, 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.

(2) Centrifuge at 10,000 x g for 2 min, transfer supernatant to a clean 1.5 mL tube.

(3) Add 250 µL Solution C2 and vortex for 5 s, incubate at 2-8°C for 5min.

(4) Centrifuge at 10,000 x g for 1 min.

(5) Transfer 600 µL supernatant to a clean 2 mL tube.

(6) Add 200 µL Solution C3 and vortex 5 s, incubate at 2-8°C for 5min.
Make sure to vortex 5 seconds, too short time will reduce DNA purity.

(7) Centrifuge at 10,000 x g for 1 min.

(8) Transfer ~750 µL supernatant to a clean 2 mL tube.

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

(10) Load 675 µl of the sample (volume: 1950 µl) 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.

(11) If the membrane is not stained brown, wash with 650 µL of 100% ethanol once and then 500 µL of solution CB twice.
If the membrane is stained, prepare a mix of 300 µL of Solution C4 and 370 µL of 100% ethanol for each sample. Wash the column with this mixture first. Follow this wash with the 100% ethanol once, and the Solution C5 twice.
For each wash, centrifuge at 10,000 x g for 30 s at room temperature. Discard the flow through.

(12) Centrifuge Spin filter one more time at 10,000g for 2 min. Carefully transfer the Spin

filter to a new 2 ml tube.
Note: The lower rim in the filters can collect Solution C5. Use a 10 µl pipette tip to withdraw any liquid from the filter interior before treatment with eluent.

(13) Add 50 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.

(14) Check DNA purity with Nanodrop. Determine the dsDNA concentration by Picogreen test ("dsDNA quantification with PicoGreen" at http://ieg.ou.edu/protocol.htm).