DNA Extraction and cleanup of bulk samples

We are using a modified version of the DNeasy PowerMax Soil Kit (Qiagen) protocol. In order to avoid the sheering of genomic DNA, we have replaced Step 4 of the DNeasy PowerMax Soil Kit protocol with an overnight proteinase K digestion as described below.

This protocol is the same for each three bulk fractions (motile 106µm - 500µm, motile 500µm - 2 mm and sessile fractions). Our standardized protocol suggests extracting 10-g of sessile matter and half of each motile fraction after decantation.

Materials:

- Falcon tubes containing 10-g of sessile fraction or half of total weight of decanted motile fractions (up to 10-g per extraction, multiply accordingly the number digestions if half the total weight of the motile fraction is >10-g)
- 1 DNeasy PowerMax Soil Kit
- Proteinase K at 20mg/mL for a final working concentration of 500 µg/-mL
- 1 DNeasy PowerClean Pro CleanUp Kit
- Sterile 200-µL, 1-mL, 5-mL, 10-mL pipettors and pipet tips
- DNA decontaminating solution (10%, DNAway, etc…)
- Marker

1- DNA extraction protocol:

1. Wear gloves at all time. Carefully clean bench station and pipettors.
2. Label the appropriate number of tubes according to DNeasy PowerMax Soil protocol.
3. Check that solutions are not precipitated. Use DNeasy PowerMax Soil protocol for troubleshooting.
4. Centrifuge falcon tubes containing the appropriate weight of bulk fraction to be extracted at 2,500 rcf for 10 min.
5. Discard the preservative (Ethanol or DMSO).
6. Add 15-mL of PowerBead Solution and vortex vigorously for 1 min.
7. Add 1.2-mL of Solution C1 and vortex vigorously for 1 min.
8. Add 405-µl of 20mg/-mL Proteinase K and incubate overnight at 56ºC in a shaking incubator. The tubes should lie as flat as possible.
9. After the digestion phase, let the tubes cool down at room temperature.
10. Centrifuge tubes at 2,500 rcf for 3 min.

From this point on, protocol is identical to DNeasy PowerMax Soil protocol

11. Transfer the supernatant to a clean, labeled Collection Tube.
12. Add 5-mL of Solution C2. Invert twice to mix. Incubate at 4°C for 10 min.
13. Centrifuge tubes at 2,500 rcf for 4 min.
14. Transfer supernatant to a clean labeled Collection Tube.
15. Add 4-mL of Solution C3. Invert twice to mix. Incubate at 4°C for 10 min.
16. Centrifuge tubes at 2,500 rcf for 4 min.
17. Transfer supernatant to a clean labeled Collection Tube.
18. Shake Solution C4 and add 30-mL of Solution C4 to supernatant. Invert twice to mix.
19. Fill labeled Spin filter with solution from step 18, centrifuge at 2,500 rcf for 2 min, and discard flow through.
20. Repeat Step 19 adjusting centrifugation time if necessary (the filter may clog and longer centrifugation time may be required).
21. Repeat Step 19 using the remainder of step 18 solution.
22. Add 10-mL of Solution C5 to spin filter and centrifuge for 3 min at 2,500 rcf.
23. Discard flow through.
24. Centrifuge spin filter for 5 min at 2,500 rcf.
25. Place spin filter in a clean labeled Collection Tube avoiding transferring solution C5 into the next step.
26. Add 5-mL of solution C6 to the center of the filter membrane.
27. Incubate at room temperature for 10 min.
28. Centrifuge for 3 min at 2,500 rcf.
29. Discard Spin Filter.

DNA can be frozen at (-20°C to -80°C) for storage or proceed to cleanup

2- DNA cleanup protocol:

This protocol is identical to the protocol provided with the DNeasy PowerClean Pro CleanUp Kit.

1. Add 100-µL of gDNA to a 2-mL labeled Collection Tube.
2. Add 50-µL of Solution DC 1 to DNA and vortex briefly.
3. Add 50-µL of Solution DC 2 and vortex briefly.
4. Centrifuge tubes at 13,000 rcf for 2 min.
5. Transfer the supernatant to a clean, labeled 2-mL Collection Tube.
6. Shake to mix Solution DC 3. Add 400-µL of Solution DC 3. Vortex to mix.
7. Centrifuge tubes briefly to remove any solution from the cap.
8. Load up to 600-µL onto Spin Filter and centrifuge at 10,000 rcf for 1 min. Discard flow through.
9. Add 500-µl of Solution DC 4 to Spin Filter and centrifuge at 10,000 rcf for 30 sec. Discard flow through.
10. Repeat step 9.
11. Centrifuge Spin Filter at maximum speed for 2 min.
12. Carefully place Spin Filter in new 2-mL labeled Collection Tube avoiding transferring any Solution DC 4 onto Spin Filter.
13. Add 90-µL of Solution DC 5 to center of white filter membrane and incubate at room temperature for 10 min.
14. Centrifuge at 10,000 rcf for 1 min.
15. Discard the Spin Filter and freeze DNA (-20°C to -80°C) for storage. DNA is now ready for downstream applications.