High Purity DNA from Soil Samples

Purifying high quality DNA from the microrganisms present in soil is a challenging goal. DNA purified from soil (microrganisms) often contains humic and fulvic acids which can inhibit various enzymes, such as Taq DNA polymerase, used in downstream assays. Fortunately, there is a well-tested solution for purifying DNA from soil samples which is accomplished with simple modifications of the MasterPure™ Complete DNA & RNA Purification Kit. The procedure employs a straightforward cell lysis while inactivating endogenous ribonucleases, followed by a rapid desalting procedure to remove contaminating molecules without the use of toxic organic solvents. The purified DNA which is free of humic and fulvic acid contaminants is suitable for many downstream applications including next gen sequencing, PCR, and fosmid library production.

The MasterPure™ Complete DNA and RNA Purification Kit (Lucigen) can be easily adapted to purify DNA from microganisms (metagenomic DNA) present in soil. The isolated, randomly sheared DNA is high molecular-weight (~40 kB), free of humic and fulvic acids, and is suitable for downstream applications including next gen sequencing, PCR and fosmid library production. For increased reliability and simplicity in fosmid library production, we recommend using the isolated DNA in conjunction with the CopyControl™ Fosmid Library Production Kit or CopyControl HTP Fosmid Library Production Kit (Lucigen). For next gen sequencing and PCR applications, we recommend using the NxSeq® UltraLow DNA Library Kit and FailSafe™ PCR Systems, respectively.

User-Supplied Reagents:

Phosphate Buffered Saline (PBS)

0.45-µm Filter membranes

1.2-µm Filter membranes

Filtration apparatus (Millipore, Cat. No. XX1004700, or equivalent)

Miracloth filtration material (Calbiochem) or sterile cheesecloth

50-mL Conical tubes

1.7-mL Microcentrifuge tubes

Tween® 20 (molecular biological grade)

Isopropanol

70% Ethanol (made from absolute ethanol)

The following products are also available:

- CopyControl™ Fosmid Library Production Kit and CopyControl™ HTP Fosmid Library Production Kit (http://www.lucigen.com/CopyControl-Fosmid-Library-Production-Kits/)

- End-It™ DNA End-Repair Kit (http://www.lucigen.com/End-It-DNA-End-Repair-Kit/)
- NxSeq® UltraLow DNA Library Kit (http://www.lucigen.com/NextGen-Seq/)
- FailSafe™ PCR Systems (http://www.lucigen.com/FailSafe-and-trade-PCR-Systems/)

Isolation of Metagenomic DNA from Soil Samples

A. Extraction

Each extraction will use 50 mL of PBS with 0.1% Tween 20. Before starting, make enough PBS with 0.1% Tween 20 for the number of samples you'll be purifying by adding 50 µL of Tween 20 per 50 mL of PBS to achieve a final concentration of 0.1% Tween 20. Note: It is critical to follow the recommended centrifugal speeds as indicated in the protocol for efficient recovery of the microbes from the soil samples.

1. Add 1 g of wet soil (or 200-300 mg of compost) to a 50-mL screw-cap conical tube and add 10 mL of PBS with 0.1% Tween 20.

2. Mix by vortexing at maximum speed for 1 minute to disperse and dissociate the soil particles.

3. Centrifuge the soil suspension at 1,600 x g for 4 minutes in a tabletop centrifuge. Pour the supernatant into a new 50-mL tube.

4. Add 20 mL of the remaining PBS with 0.1% Tween 20 (per extraction) to the original soil pellet and mix by vortexing at maximum speed for 1 minute.

5. Centrifuge the soil suspension at 900 x g for 3 minutes in a tabletop centrifuge. Important! Do not exceed 900 x g. Combine the supernatant with the previously collected supernatant.

6. Reextract the soil pellet again by repeating steps 4 and 5 (above), and then proceed to step 7 (below).

7. Briefly centrifuge the pooled supernatant at 900 x g for 2 minutes in a tabletop centrifuge and transfer the supernatant to a fresh 50-mL tube.

B. Filtration, Lysis, and Protein Precipitation

1. Pour the entire collected supernatant (50 mL) through four layers of Miracloth filtration material (Calbiochem).

2. Prefilter the sample through the 1.2-µm filter membrane using an appropriate filterware apparatus (e.g., Millipore, Cat. No. XX1004700). Collect the filtrate.

3. Pass the collected filtrate through the 0.45-µm filter membrane using an appropriate filterware apparatus in order to trap the microbial mass on the filter. Retain the filter membrane.

4. Using forceps and scissors presoaked in 70% ethanol, remove the membrane from the filter apparatus, cut the membrane in half, and place each half (rounded side down) along the side (near the bottom) of a 50-mL sterile conical tube. The upper surface of the filter needs to face the center (not wall) of the tube. Do not allow the filter membrane to dry out.

5. Prepare the Filter Wash Buffer by adding 1.5 µL of Tween 20 to 1.5 mL of PBS immediately before use. Add 1.5 mL of Filter Wash Buffer containing 0.1% Tween 20 to the filter pieces in the tube.

6. Vortex the tube at the low speed setting to rewet the filter pieces, then increase the setting to the highest speed.

7. Transfer the cell suspension to a clean microcentrifuge tube, then centrifuge the tube at 14,000 x g or top speed in a microcentrifuge for 2 minutes to pellet the cells. Discard the supernatant.

8. Resuspend the cell pellet in 300 µL of TE Buffer, then add 2 µL of Ready-Lyse Lysozyme Solution and 1 µL of RNase A to the cell suspension. Mix, and centrifuge briefly.

9. Incubate the tube at 37°C for 30 minutes.

10. Add 300 µL of 2x Tissue and Cell Lysis Solution and 1 µL of Proteinase K to the tube. Mix by vortexing.

11. Briefly pulse-centrifuge the tube to ensure that all of the solution is in the bottom of the tube.

12. Incubate at 65°C for 15 minutes.

13. Place on ice for 3-5 minutes.

14. Add 350 µL of MPC Protein Precipitation Reagent to the tube and mix by vortexing vigorously for 10 seconds.

15. Pellet the debris by centrifuging for 10 minutes at 20,000 x g or maximum speed, in a microcentrifuge at 4°C.

16. Transfer the supernatant to a clean 1.7-mL microcentrifuge tube and discard the pellet.

17. Add 570 µL of isopropanol to the supernatant. Mix by inverting the tube several times.

18. Pellet the DNA by centrifuging for 10 minutes at 20,000 x g or maximum speed, in a microcentrifuge at 4°C.

19. Use a pipet tip to remove the isopropanol without dislodging the DNA pellet. Briefly pulse-centrifuge the sample and remove any residual liquid with a pipet tip, without disturbing the pellet.

20. Add 500 µL of 70% ethanol to the pellet without disturbing the pellet. Then centrifuge for 5 minutes at 20,000 x g or maximum speed, in a microcentrifuge at 4°C.

21. Use a pipet tip to remove the ethanol without dislodging the DNA pellet. Briefly pulse-centrifuge the sample and remove any residual liquid with a pipet tip without disturbing the pellet.

22. Air-dry the pellet for 8 minutes at room temperature. Note: Do not over-dry the DNA pellet.

23. Resuspend the DNA pellet in 40 µL of TE Buffer.

24. Validate the size and concentration of the isolated DNA by gel electrophoresis on a 1% agarose gel.

The isolated DNA is ready for PCR, next gen sequencing, or for end-repair and subsequent cloning into a vector for construction of a fosmid library. If using the CopyControl Fosmid Library Production Kit (Lucigen), proceed with end-repair (Section B) and ligation (Section E). The protocol can be found at

http://www.lucigen.com/docs/manuals/MA171E-CopyControl-Fosmid-library-production-kits.pdf

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