

Purification of soil metagenomic DNA with the MasterPure™ Complete Kit

INTRODUCTION

Studying the rich diversity of the soil metagenome can prove challenging, as most bacteria are unculturable and many substances present in soil are inhibitory to downstream applications. The MasterPure™ Complete DNA and RNA Purification Kit can be easily adapted to purify metagenomic DNA from microorganisms present in soil. The isolated, randomly sheared DNA is high molecular weight (~40 kB), free of humic and fulvic acids, and 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](#). For next gen sequencing and PCR applications, we recommend using the [NxSeq® UltraLow DNA Library Kit](#) and [FailSafe™ PCR Systems](#), respectively.

METHODS

Use the reagents from the MasterPure Complete DNA & RNA Purification Kit. You will need to supply the following additional reagents:

- Phosphate-buffered saline (PBS)
- Ready-Lyse™ Lysozyme Solution ([Lucigen Cat. No. R1804M](#))
- 0.45-µm filter membranes (e.g., Sterlitech Cat. No. CA04547100)
- 1.2-µm filter membranes (e.g., Sterlitech Cat. No. CA1247100)
- Filtration apparatus (Millipore Sigma Cat. No. XX1004700, or equivalent)
- Miracloth filtration material (Millipore Sigma) or sterile cheesecloth
- 50 mL conical tubes
- 1.7 mL microcentrifuge tubes
- Tween® 20 (molecular biology grade)
- Isopropanol
- 70% ethanol (prepared from absolute ethanol)

Extraction

Each extraction will use 50 mL of PBS with 0.1% Tween 20. Before starting, prepare enough buffer for the number of samples you will 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, then 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 $1,600 \times g$ 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 $900 \times g$ for 3 minutes in a tabletop centrifuge. **Important!** Do not exceed $900 \times g$. Remove the supernatant and combine it with the previously collected supernatant from step 3.
6. Re-extract the soil pellet again by repeating steps 4 and 5, and then proceed to step 7.
7. Briefly centrifuge the pooled supernatant at $900 \times g$ for 2 minutes in a tabletop centrifuge. Transfer the supernatant to a fresh 50 mL tube.

Filtration, Lysis, and Protein Precipitation

1. Pour the entire collected supernatant (50 mL) through four layers of Miracloth or sterile cheesecloth.
2. Prefilter the sample through the 1.2 µm filter membrane using an appropriate filter apparatus. Collect the filtrate.
3. Pass the collected filtrate through the 0.45 µm filter membrane using an appropriate filter 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 the 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 $14,000 \times g$ 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 $20,000 \times g$, 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 $20,000 \times g$, 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 without disturbing the pellet. Centrifuge for 5 minutes at $20,000 \times g$, 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 electrophoresis on a 1% agarose gel.

The isolated DNA is ready for PCR, next gen sequencing, or end-repair and subsequent cloning into a vector for construction of a fosmid library. If using the CopyControl Fosmid Library Production Kit, proceed with end-repair (Section B) and ligation (Section E) in the supplied protocol ([#MA171E: CopyControl Fosmid Library Production Kits](#)).

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