Purification of metagenomic DNA from environmental water samples with the MasterPure™ Complete Kit

Introduction

Environmental water samples often harbor a complex array of microorganisms. Metagenomic analysis of such samples is complicated by the relatively large sample processing volume and the unculturable nature of most environmental bacteria. The MasterPure™ Complete DNA and RNA Purification Kit can be easily adapted to purify metagenomic DNA from water samples. The isolated, randomly sheared DNA is high molecular weight (~40 kB) 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)
- 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)

Filtration, Lysis, and Protein Precipitation

Each purification will use 1.5 mL of PBS with 0.1% Tween 20. Before starting, prepare enough buffer for the number of samples you will be purifying by adding 1.5 µL of Tween 20 per 1.5 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 water samples.

1. To remove debris from the collected water sample (100 mL), pour the water through Miracloth filtration material or sterile cheesecloth. Alternatively, centrifuge the sample at 1,000 × *g* for 5 minutes. Collect the water in a sterile container.

2. Filter the water (100 mL) 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.

3. 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.

4. 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.

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

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

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7. 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.

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

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

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

11. Incubate at 65°C for 15 minutes.

12. Place on ice for 3–5 minutes.

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

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

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

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

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

18. 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.

19. Add 500 µL of 70% ethanol without disturbing the pellet. Centrifuge for 5 minutes at 20,000 × *g*, or maximum speed, in a microcentrifuge at 4°C.

20. 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.

21. Air-dry the pellet for 8 minutes at room temperature.

    Note: Do not over-dry the DNA pellet.

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

23. 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).