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Meta-G-Nome™ DNA Isolation Kit

Cat. No. MGN0910

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1. Introduction

The Meta-G-Nome™ DNA Isolation Kit is designed to isolate randomly-sheared, high-molecular-weight (HMW) metagenomic DNA, free of humic and fulvic acid, directly from unculturable or difficult-to-culture microbial species present in environmental water or soil samples. The DNA isolated using this kit is approximately 40 kb in size and is ready for immediate use in end-repair reactions and subsequent cloning into fosmid vectors. This protocol streamlines standard fosmid library construction by eliminating the traditional mechanical shearing and size-selection steps. Additionally, the DNA can be used as a substrate for PCR or sequencing.

For increased reliability and simplicity, we recommend using the isolated DNA in conjunction with Epicentre's CopyControl™ Fosmid Library Production Kit or CopyControl HTP Fosmid Library Production Kits.

2. Kit Contents

Desc.	Concentration	Quantity
Meta-G-Nome™ DNA Isolation Kit Contents		
The Meta-G-Nome DNA Isolation Kit provides sufficient reagents & filter membranes for DNA isolations from 10 samples:		
Extraction Buffer		500 ml
Filter Wash Buffer		15 ml
Meta-Lysis Solution (2X)*		3 ml
MPC Protein Precipitation Reagent		3.5 ml
Proteinase K	(50 µg/µl)	10 µl
Fosmid Control DNA	(100 ng/µl)	10 µl
RNase A	(5 µg/µl)	10 µl
Ready-Lyse™ Lysozyme Solution		20 µl
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)		3.5 ml
0.45-µm Filter Membranes		10
1.2-µm Filter Membranes (for soil extractions)		10

***Note:** The Meta-Lysis Solution (2X) may form a precipitate during shipment or storage. If this occurs, heat the bottle at 37°C until the precipitate dissolves. Mix thoroughly and cool to room temperature.*

Required materials not supplied in the kit:

Filtration apparatus (Millipore 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)

3. Product Specifications

Storage: Store the MPC Protein Precipitation Reagent, Proteinase K, Ready-Lyse™ Lysozyme, RNase A, and Fosmid Control DNA at –20°C in a freezer without a defrost cycle. Store the remainder of the kit components at room temperature.

Storage Buffer: Proteinase K and Ready-Lyse Lysozyme Solution are supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 10 mM $CaCl_2$, 0.1% Triton® X-100, and 1 mM dithiothreitol. RNase A is supplied in a 50% glycerol solution containing 25 mM sodium acetate (pH 4.6).

Quality Control: The Meta-G-Nome DNA Isolation Kit is function-tested by purifying DNA from 1 g of garden soil, and from known concentrations of diluted *E. coli* in a water sample. The size of the DNA is compared with a 40-kb linear control DNA on a 1% agarose gel. DNA quality is assayed by PCR.

4. Related Products

The following products are also available:

- CopyControl™ Fosmid Library Production Kit
- CopyControl™ HTP Fosmid Library Production Kit
- Direct Lysis Fosmid96 DNA Isolation Kit
- End-It™ DNA End-Repair Kit

5. Isolation of Metagenomic DNA from Water Samples

1. To remove debris from the collected water sample (100 ml), pour the water through Miracloth filtration material (Calbiochem), sterile cheesecloth, or centrifuge at 1,000 x g for 5 minutes and collect the water in a sterile container.
2. Filter the water (100 ml) through the 0.45-µm filter membrane (provided) to trap microbes onto the filter, using the appropriate filtration apparatus (e.g., Millipore, catalog no. XX1004700).
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 wall) of the tube. Do not allow the filter membrane to dry out.
4. Prepare the Filter Wash Buffer by adding 2 µl of Tween 20 to 1 ml of Filter Wash Buffer immediately before use. Add 1 ml of Filter Wash Buffer containing 0.2% Tween 20 to the filter pieces in the tube to wash off the microbes trapped on the membrane.
5. Vortex the tube at the low speed setting to rewet the filter pieces, then increase the setting to the highest speed for ~2 minutes with intermittent breaks.
6. Transfer the cell suspension to a clean microcentrifuge tube, then centrifuge the tube at 14,000 x g for 2 minutes to pellet the cells. Discard the supernatant.
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 by vortexing.
8. Incubate at 37°C for 30 minutes.

9. Add 300 µl of Meta-Lysis Solution (2X) 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. Cool to room temperature, then 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 centrifugation for 10 minutes at 20,000 x g or at 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 centrifugation for 10 minutes at 20,000 x g or at 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 to the pellet without disturbing the pellet. Then centrifuge for 5 minutes at 20,000 x g or at 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 50 µl of TE Buffer.
23. Validate the size and concentration of the isolated DNA by comparison to the Fosmid Control DNA (40 kb; 100 ng/µl) provided in the kit, via gel electrophoresis on a 1% agarose gel. Use 2 µl of the isolated DNA Preparation for this analysis.

The isolated DNA is ready for PCR or for end-repair and subsequent cloning into a vector for construction of a fosmid library. If using Epicentre's CopyControl Fosmid Library Production Kit, proceed with end-repair (Part B) and ligation (Part E). The protocol can be found at http://www.epicentre.com.

For rapid Preparation of template DNA for sequencing, use Epicentre's Direct Lysis Fosmid96 DNA Purification Kit.

6. Isolation of Metagenomic DNA from Soil Samples

A. Extraction

Before starting, add Tween 20 to the Extraction Buffer (1 µl Tween 20 per milliliter of Extraction Buffer) to achieve a final concentration of 0.1% Tween 20. Each extraction will use 50 ml of Extraction Buffer with 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 Extraction Buffer with 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 remaining Extraction Buffer with Tween 20 (per extraction) to the 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), 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 collected supernatant (50 ml) through four layers of Miracloth filtration material (Calbiochem).
2. Prefilter the sample through the 1.2-µm filter membrane provided using an appropriate filterware apparatus (e.g., Millipore, catalog no. XX1004700). Collect the filtrate.
3. Pass the collected filtrate through the 0.45-µm filter membrane provided 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 Filter Wash Buffer 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 for ~2 minutes to wash off the microbes trapped on the filter membrane.

7. Transfer the cell suspension to a clean microcentrifuge tube, then centrifuge the tube at 14,000 x g for 2 minute 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 to bring all of the solution to the bottom of the tube.
9. Incubate at 37°C for 30 minutes.
10. Add 300 µl of Meta-Lysis Solution (2X) 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. Cool to room temperature, then 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 centrifugation for 10 minutes at 20,000 x g or at 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 centrifugation for 10 minutes at 20,000 x g or at 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 at 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 comparison to the Fosmid Control DNA (40 kb; 100 ng/µl) provided in the kit, via gel electrophoresis on a 1% agarose gel. Use 5 µl of the isolated DNA Preparation for this analysis.

The isolated DNA is ready for PCR or for end-repair and subsequent cloning into a vector for construction of a fosmid library. If using Epicentre's CopyControl Fosmid Library Production Kit, proceed with end-repair (Section B) and ligation (Section E). The protocol can be found at http://www.epicentre.com.

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