Nanotrap® Microbiome A; 10 mL Manual Protocol with Promega Maxwell® HT Environmental TNA Kit

Objective: This protocol uses Nanotrap Microbiome A Particles and Nanotrap Enhancement Reagent 2 to capture and concentrate microbes in environmental water samples. It is optimized for microbe capture from 10 mL samples and is compatible with Promega Maxwell® HT Environmental TNA Kit

Materials and equipment:

Sample Type	
Environmental Water Samples	
Concentration Reagent	**Vendor**
Nanotrap® Microbiome A Particles	Ceres Nanosciences; SKU# 44202
Nanotrap® Enhancement Reagent 2 (ER2)[1]	Ceres Nanosciences; SKU# 10112
Extraction Kit	**Vendor**
Promega Maxwell® HT Environmental TNA Kit	Promega; Cat# AX9190
Materials/Equipment	**Vendor**
Heat Block	Southern Labware; SKUBSH200
Mini Centrifuge	Scientific Industries; SKU WZ-MF6000
DynaMag™-15 Magnet	Thermo Fisher Scientific; Cat# 12301D
DynaMag™-2 Magnet	Thermo Fisher Scientific; Cat# 12321D
15 mL Conical Centrifuge Tubes	Stellar Scientific; SKU T15-100
Tube Rotator	Stellar Scientific; SKU BS-RTMNI-2
Serological Pipettes and Controller	Fisherbrand; Cat# 13-678-11E
2mL Micro Centrifuge tubes	Stellar Scientific; SKU T20-100
Mini Vortex Mixer	Scientific Industries; SKU SI-236
General Reagents	**Vendor**
Ethanol	Decon™ Laboratories Decon Labs; # 3916EA
Isopropanol	Fisher Scientific; BP26184
Molecular Biological Grade Water	Corning; Cat# 46-000-CM

[1] Precipitate can form in ER2 if stored below room temperature. Allow ER2 to return to room temperature to dissolve the precipitate (can invert 2-3 times to help resuspend, do not heat).

Capture and Extract Microbes using Nanotrap Microbiome Particles

Procedure:

1. **Nanotrap Microbiome A Promega Manual Procedure-Part 1**
    1. Invert the environmental water sample 5 times to mix. Then, let it sit for 45 seconds at room temperature. (No need to wait for sample to reach room temperature before processing)
    2. Add 10 mL of environmental water sample into a clean 15 mL conical tube.
    3. Add 100 µL of Nanotrap Enhancement Reagent 2 (ER2) to the sample and then invert 2 times to mix it.
    4. Add 150 µL of Nanotrap Microbiome A Particles to the sample and then invert 2 times to mix the particles.
    5. Incubate samples with Nanotrap particles at room temperature for 10 minutes.

        Note: Invert every 5 minutes or use a rotator.
    6. Place the tube on a DynaMag-15 magnetic rack to separate the Nanotrap particles from the sample for 5 minutes.
    7. Using a serological pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

        Note: Can use a P-1000 or P-200 pipette to remove any remaining supernatant from the sample (be careful to not lose any Nanotrap particles when removing supernatant).
    8. Add 1 mL of molecular grade water to the tube and re-suspend the Nanotrap particle pellet by pipetting on the walls of the conical tube, gently re-suspend until all Nanotrap particles have been completely collected.
    9. Transfer the Nanotrap particle suspension to a new 2 mL microcentrifuge tube.
    10. Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.
    11. Using a P-1000 pipette, discard the supernatant carefully without disturbing the Nanotrap particle pellet.

        Note: If any small amount of liquid is still present, use a smaller pipette to remove all the supernatant from the bottom of the tube.
    12. Add 300 µL of Cell Lysis Solution to Nanotrap particle pellet, pipette up and down until Nanotrap particles are resuspended completely.
    13. Close the tube lid, incubate the samples at RT for 10 minutes.
    14. Place the 2 mL microcentrifuge tube on a DynaMag-2 magnetic rack to separate the Nanotrap particles from the sample for 2 minutes.

Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops from inside the lid before magnetic separation.

15. Transfer supernatant/lysate to a new 2 mL collection tube and discard the Nanotrap particles pellet.
16. Sample is now ready for Part 2.

2. **Nanotrap Microbiome A Promega Manual Procedure-Part 2.**
    1. Add 50 µL of Alkaline Proteinase to the sample/lysate.
    2. Add 400 µL of 100% isopropanol to the sample/lysate.
    3. Add 35 µL of Magnetic Beads Resin to the sample/lysate.
    4. Vortex to mix, then incubate at RT for 20 minutes.
        1. Place on shaker/rotator.
    5. Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant using a pipette.

        Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops from inside the lid before magnetic separation.
    6. Add 1000 µL of Dilute Wash Solution to sample and re-suspend the magnetic beads using a pipette.
    7. Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant.
    8. Add 1000 µL of Dilute Wash Solution to sample and re-suspend the magnetic beads using a pipette.
    9. Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant.
    10. Add 450 µL of 50% EtOH to sample and re-suspend the magnetic beads using a pipette.
    11. Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant by using a pipette.
    12. Add 450 µL of 50% EtOH to sample and re-suspend the magnetic beads using a pipette.
    13. Place the tube on a DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes, then discard the supernatant by using a pipette.
    14. Centrifuge the tube (Mini Centrifuge at 2000 g for 30 seconds).
    15. Place the tube on a DynaMag-2 magnetic rack, then remove excess 50% EtOH using a smaller pipette.

16. Open caps, allow samples to air dry at room temperature for 10 minutes.
17. Add 50 µL of Tris-HCL (pH 8.0) to re-suspend the magnetic beads and then incubate at RT for 7 minutes (close caps).
    1. Place on shaker
18. Place the tube in the DynaMag-2 magnetic rack to separate the magnetic beads from the sample for 2 minutes.

    Note: May need to briefly centrifuge the tube (Mini Centrifuge at 2000 g for 2-5 seconds) to remove drops/condensation from inside the lid before magnetic separation.
19. Transfer the supernatant to a new tube, the sample is ready for downstream analysis or can be stored at -80ºC.

    Note: Multiple freeze-thaw cycles may cause degradation.