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Sterivex DNA Extraction with PowerSoil Kit

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ABSTRACT

Adapted from: Jacobs, J., Rhodes, M., Sturgis, B. & Wood, B. 2009. Influence of environmental gradients on the abun- dance and distribution of Mycobacterium spp. in a coastal lagoon estuary. Appl. Environ. Microbiol., 73787384, DOI: 10.1128/AEM.01900-09

This protocol was used to extract DNA from sterivex filters collected during time series sampling in Singapore from 2017-2019 at Kusu and Hantu islands. The protocol follows instructions from the Qiagen Powersoil kit with the addition of phenol-chloroform-isoamyl alcohol between solutions C1 and C2.

PROTOCOL CITATION

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MATERIALS TEXT

Qiagen Power Soil DNA Exraction kit
Phenol-chloroform-isoamyl alcohol

SAFETY WARNINGS

Use phenol-chloroform-isoamyl alcohol in fume hood

DNA extraction of sterivex filter using PowerSoil kit

1 Thaw sterivex samples and remove any RNAlater or excess water from filter using a syringe.

2 Using sterile pliers, break open sterivex by applying pressure along the seam of the casing. Do so over a sterile space as the filter can pop out quickly.

3 Using sterile razor blade, slice the filter into 6 lengthwise strips. Place strips into powersoil bead tube.

4 Add 60µL of solution C1 and vortex briefly. Incubate at 70°C and 500rpm for 10 minutes.

5 Vortex at max speed. Re-incubate at 70◦C and 500rpm for 10 minutes.

6 In fumehood, add 700µL of room temperature phenol-chloroform-isoamyl alcohol to dissolve filter. Vortex at max speed for 10 minutes. (Steps 6, 8,9, 11, 12 should be done in a fumehood).

7 Centrifuge at 10,000g for 30 seconds.

8 Transfer 800µL supernatant to a clean 2mL collection tube.

9 Add 250µL solution C2, vortex for 5 seconds, incubation at 4°C for 5 minutes.

10 Centrifuge at 10,000g for 1 minute at room temperature.

11 Transfer 600µL supernatant to a clean 2mL collection tube.

12 Add 200µL of solution C3, vortex briefly. Incubate at 4°C for 5 minutes.

13 Centrifuge at 10,000g for 1 minute at room temperature.

14 Transfer 750µL supernatant to a clean 2mL collection tube.

15 Add 1200µL of solution C4 (shake to mix before use). Vortex for 5 seconds.

16 Load 675µL into spin filter, centrifuge at 10,000g for 1 minute at room temperature, discard flow through; add 675µL again to spin filter, centrifuge at 10,000g for 1 minute at room temperature, discard flow through. Load remaining supernatant into spin filter and centrifuge at 10,000g for 1 minute at room temperature, discard flow through.

17 Add 500µL of solution C5, centrifuge at 10,000g for 30 seconds at room temperature.

18 Discard flow through

19 Centrifuge again at 10,000g for 1 minute at room temperature.

20 Carefully place spin filter in a clean 2ml collection tube.

21 Add 60µL of nuclease free water to the center of the white filter membrane. (Adjust volume of nuclease free water based on expected yield and desired DNA concentration). Let sit for 5 minutes.

22 Centrifuge at 10,000g for 30 seconds at room temperature.

23 Discard spin filter. Aliquot DNA for Qubit and PCR. Store remaining sample at -20°C for short term storage or -80°C for long term storage.