Protocol for extracting eDNA from water samples using Qiagen's DNeasy PowerWater Kit®

This is a protocol for extracting eDNA from water samples using Qiagen's DNeasy PowerWater Kit® (Cat. No. / ID: 14900-100-NF). It is taken from Qiagen's online short protocol (link below), modified with notes for use by our lab.

Before Starting:

- You should have filtered 1 L or more of water prior to starting this protocol. You will begin here with the filter membrane containing the filtrate.
- Some of the kit solutions have to be prepared for use:
    - Solution PW1 must be warmed at 55°C for 5–10 minutes to dissolve precipitates prior to use. Solution PW1 should be used while still warm.
    - If Solution PW3 has precipitated, heat at 55°C for 5–10 minutes to dissolve precipitate.
    - Shake to mix Solution PW4 before use.

Procedure:

1. Using sterile membrane (flat-tipped) forceps, pick up the filter membrane and roll the filter into a cylinder with the top side facing inward.
    a. Note: Do not tightly roll or fold the filter membrane.
    b. Video at: www.mobio.com.

2. Insert the filter into a 5 ml PowerWater DNA Bead Tube.

3. Add 1 ml of Solution PW1 to the PowerWater DNA Bead Tube.
    a. Note: For samples containing organisms that are difficult to lyse an additional heating step can be included. See Qiagen's Alternate Lysis Method in the Hints and Troubleshooting Guide.

4. Secure the tube to a vortex adapter and vortex at maximum speed for 5 min.
    a. Note: Centrifuging the tubes ≤ 4000 x g for 1 min at room temperature will help you collect more of the supernatant but this step can be skipped if an adequate centrifuge is not available. *(There is one in McGS 222)*

5. Transfer the supernatant to a clean 2 ml collection tube (provided). Draw up the supernatant using a 1 ml pipette tip by placing it down into the beads.
    a. Note: Placing the pipette tip down into the beads is required. Pipette until you have removed all the supernatant. Expect to recover 600–650 µl of supernatant.

6. Centrifuge at 13,000 x g for 1 min at room temperature.

7. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
8. Add 200 µl of Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.
9. Centrifuge the tubes at 13,000 x g for 1 min.
10. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube (provided).
11. Add 650 µl of Solution PW3 and vortex briefly to mix.
12. Load 650 µl of supernatant onto a MB Spin Column. Centrifuge at 13,000 x g for 1 min. Discard the flow-through. Repeat until all the supernatant has been processed.
13. Place the MB Spin Column Filter into a clean 2 ml collection tube (provided).
14. Add 650 µl of Solution PW4 (shake before use). Centrifuge at 13,000 x g for 1 min.
15. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x g for 1 min.
16. Discard the flow through and centrifuge again at 13,000 x g for 2 min.
17. Place the MB Spin Column into a clean 2 ml collection tube (provided).
18. Add 100 µl of Solution EB to the center of the white filter membrane.
19. Centrifuge at 13,000 x g for 1 min.
20. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Notes:

- The DNeasy PowerWater Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label. It comes in 50 or 100 sample boxes.
- Link to Qiagen quickstart protocol: https://www.qiagen.com/us/resources/resourcedetail?id=58f43d7e-172a-4970-84f0-9cb335a8d262&lang=en
- Link to product handbook: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powerwater-kit/