DNA Extraction Method for Rooney-Varga Samples

Sample collection:

1. Seawater was collected using polypropylene buckets rinsed twice with 70% ethanol and with seawater from the sampling location
2. Seawater was successively filtered through 100 µm diameter pore-size membrane filters to remove large particulates (e.g., zooplankton and marine snow, which were not analyzed), and subsequently filtered through 5 µm and 0.22 µm pore size-filters to collect attached and free-living bacteria, respectively.
3. Filtration was conducted at sea immediately after sample collection and filters were frozen on dry ice and held at -70˚C until analyzed.

Materials:

FastDNA Spin Kit for Soil (MP Biomedicals Inc., Solon OH, USA)

DNA extraction:

1. Cut either one-half or a whole filter into ~6 x 6 mm squares using a sterile razor blade
2. Transfer filter pieces to a Lysing Matrix E Tube using sterile forceps.
3. Add 978 µL sodium phosphate buffer and 122 µL MT buffer to the Lysing Matrix E Tube.
4. Lyse cells using a Mini-Bead-Beater at maximum speed for 30 seconds, then place on ice to cool, and repeat two more times.
5. Freeze-thaw tube in liquid nitrogen and 65 ˚C to improve cell lysis after bead-beating.
6. Centrifuge the Lysing Matrix E tube at 14,000 x *g* for 30 seconds.
7. Transfer the supernatant to a clean, sterile microfuge tube.
8. Add 250 µl PPS reagent and mix tube by shaking by hand ten times.
9. Centrifuge at 14,000 x g for five minutes to pellet precipitate.
10. Transfer supernatant to a clean, sterile 15 ml conical tube
11. Add 1 ml Binding Matrix Suspension to the supernatant.
12. Place on a rotator for 2 minutes to allow binding of DNA to matrix.
13. Place tube in a rack for 3 minutes to allow settling of matrix.

14. Remove 700 µL supernatant and discard. Resuspend remaining Binding Matrix in remaining supernatant.
15. Transfer ~600 µL of mixture to a SPIN™ Filter and centrifuge at 14,000 x g for 1 minute. Empty the Catch tube and add the remaining supernatant-binding matrix suspension to SPIN™ Filter and repeat centrifugation. Discard flow-through.
16. Add 500 µL SEWS-M to the SPIN™ Filter and centrifuge at 14,000 x g for 1 minute. Discard flow-through and replace SPIN™ Filter in Catch tube. Centrifuge at 14,000 xg for 2 minutes to remove residual SEWS-M from matrix.
17. Remove SPIN™ Filter and place in fresh Catch Tube. Air dry for 5 minutes at room temperature in PCR hood.
18. Add 50 µL molecular biology grade water and gently stir matrix on filter membrane with a pipette tip.
19. Centrifuge at 14,000 x g for 1 minute to transfer eluted DNA to a Catch Tube.
20. In order to ensure that all alcohol has been removed from samples, lyophilize samples in Speed Vac for ~20 minutes on medium heat setting. Resuspend DNA in 50 µL molecular biology grade water.