Project WiCCED Microbiome Sampling Protocol

Reproducibility starts with sample collection and preservation. If you are unsure about whether or not you want to analyze samples, it is best to preserve them immediately and decide later. This protocol enables you to do that and assumes that you will be doing your own chemical/physical analyses on the site or experiment. Following this protocol has proven to provide extremely low levels of contaminants in mock samples and produce enough DNA for multiplex amplicon sequencing, Illumina shotgun metagenomic sequencing, and potentially PacBio long read sequencing. Questions? Please contact us at: microbiome@udel.edu.

Materials Needed for Each Sample

- Sterile pipette for water or loose sediment (SAMCO 202 is what we provide in kits)
- Sterile corer or spatula for soils or firm sediments (15 mm 2093-00 Humboldt Mfg on Amazon with a 4" ½-⅜" zinc plated bolt to push the core out. These can be cleaned and autoclaved repeatedly, wrap in foil or a sealed autoclave envelope)
- Sterile graduated 50 mL centrifuge tube with 20 mL DESS (recipe below, tubes e.g. Fisher 14-375-150)
- Gloves (nitrile exam gloves)
- Isopropanol wipes (Fisher 22-363-750 or equivalent)

Protocol

1. Put on a pair of gloves and wipe them down with an alcohol wipe.
2. To sample soil or firm sediment (thick mud, beach sand):
    a. Brush away as much plant material as possible from the surface
    b. Unwrap the corer and grab it by the T shaped end
    c. Plunge the corer vertically into the ground (or core if sub-sampling) as far as it will easily go
    d. Pull it back out and empty it into the tube with DESS
        i. Use the bolt to push the sample out of the corer
        ii. **Repeat until DESS + sample volume = 40 ml**
        iii. Mix well
        iv. Use the corer as a scoop when the corer can't plunge into rocky soil or sample repeatedly falls out before transferring; alternatively, see step 8
3. To sample liquid or loose sediment (thin mud or muck)
    a. Unwrap the pipette and grab it at the bulb end
    b. Use the bulb to suck up liquid and transfer it to the tube **with preservative**
        i. Repeat until DESS + sample volume = 40 ml
    c. If sample is too thick for the pipette, see step 2 (unless already attempted)
4. Make sure the tubes are tightly capped. For shipping, place in secondary containment, i.e. sealed in ziploc bags with absorbent material in a sturdy box.
5. Store long term at 4 C. **DO NOT FREEZE!**

DESS Recipe:
Adapted from Beknazarova, M., Millsteed, S., Robertson, G., Whiley, H., and Ross, K. (2017) Validation of DESS as a DNA Preservation Method for the Detection of *Strongyloides* spp. in Canine Feces. *Int J Environ Res Public Health* **14.**:624.

1. Weigh out 52.8 g disodium EDTA
2. Add to 100 ml nanopure water with stir bar
3. Add 1 M NaOH while monitoring pH, EDTA will dissolve.
4. When pH is 7.5 and all EDTA has dissolved, adjust volume to 400 mL
5. Add 100 ml of DMSO
6. Pour into bottle containing 50 g NaCl
7. Autoclave (20‘, 15 psi, 121 C)
8. After cooling, aliquot into sterile tubes avoiding NaCl at bottom.

The final concentration is 0.25 M EDTA, 20% DMSO, sat. NaCl, pH = 7.5

References:
Tatangelo, V., Franzetti, A., Gandolfi, I., Bestetti, G., and Ambrosini, R. (2014) Effect of preservation method on the assessment of bacterial community structure in soil and water samples. *FEMS Microbiol Lett* **356**: 32–38.

Yoder, M., Wm King, I., De Ley, I.T., Mann, J., Mundo-Ocampo, M., Poiras, L., et al. (2006) DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* **8**: 367–376.